Plant Physiology

A Textbook for Colleges and Universities

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TO

E. N. T.

H. C. S. AND B. W. W.

who have broadened

horizons for so many

PREFACE

This book has developed largely from the courses in plant physiology as taught by the authors at the Ohio State University and the University of North Carolina. The authors have attempted to organize a discussion of the fundamental facts and principles of this subject which can be included between the covers of a volume of moderate dimensions. It is believed that this book can be readily adapted to use with any introductory course in plant physiology based upon prerequisites of general botany and general chemistry. The introductory course in plant physiology at the Ohio State University differs considerably from that at the University of North Carolina. In the former institution, for example, it is a two-quarter course; in the latter a one-quarter course. Nevertheless the authors expect to use this book successfully in both these courses. Although the discussion is necessarily concise the subject has been treated comprehensively, and it is believed that few if any topics which would be considered significant by the majority of plant physiologists have been neglected.

This text can be used as the basis for a conventional recitation course, or as a background source of information for student reading in connection with lecture-discussion courses. There is a continuity of presentation from chapter to chapter which especially adapts the book to such a usage. If only certain topics are selected for laboratory or lecture consideration, reading of the intervening chapters should help the student to fit the classroom work into a coordinated picture of the science as a whole.

We have attempted throughout the text to bring into bold relief the fundamental principles of plant physiology rather than to present only an encyclopedic compilation of undigested and sometimes contradictory facts. Most of the discussion is based directly on data selected from the original literature much of which is presented in tabular or graphical form. A consistent attempt has been made to keep the discussion abreast of modern developments in plant physiology without neglecting concepts which have stood the test of time.

Nowadays it is scarcely less than axiomatic that any discussion of physiology must be grounded on the principles of physics and chemistry. Furthermore, the student must have a clear concept of the physical nature of the

factors in the environment of plants if he is to attain any real insight into the mechanism of their influence upon plant processes. Hence several of the earlier chapters in this book have been devoted to a review and extension of physico-chemical principles with which the student of physiology should be familiar, and additional discussions of pertinent facts and principles of physics and chemistry have been introduced as needed in the interpretation of plant processes. Likewise the structure of organs, tissues, and cells has been described at appropriate places as necessary background information. An integrated picture of structure and process is usually necessary for a clear concept of any phase of the physiological activity of plants.

When conflicting evidence is presented some attempt at evaluation has usually been made. We feel that the student is entitled to the considered views of the authors when contradictory data are cited, as well as a chance to acquire some feeling for the weighing of evidence which is an important procedure in the methodology of science. Probably none of our colleagues will agree with every single one of our evaluations, and it is likely that some of the viewpoints which have been espoused will ultimately be shown to be erroneous.

A short list of textbooks and monographs suitable for collateral reading has been appended to each chapter. There is also a selected bibliography of journal references at the end of most of the chapters. Every paper cited in the text or from which data in tabular or graphical form have been taken is listed in the appropriate bibliography. No pretense is made of including a comprehensive list of papers on any subject. We have merely attempted to blaze a few trails into the original literature for the occasional student who wishes to follow them on his own initiative or for those teachers who wish to make a definite point of training students to look to the journal literature as the original source of information. Citation of any paper is not necessarily meant to imply that it is one of the outstanding papers in its field, nor is it ever intended to indicate priority for that contribution. Other criteria have guided us in the selection of many of the papers listed. An impartial review, for example, can often be read by students with greater profit than the most meritorious original contribution. Papers published in widely circulated journals have usually been given preference over those which have appeared in journals which are not likely to be available in many institutions. While we have not hesitated to include some foreign language citations, in general preference has been given to original literature appearing in the English language.

A representative list of questions has been appended to most of the chapters. These are principally of the "problem" type and are especially valuable for

the catalysis of class discussions. Many have been deliberately chosen to extend the classroom discussion to applications which have not been considered in the text. There are a few to which only hypothetical answers can be given, but questions of this type can often be made most useful in stimulating class discussions. We recommend that each teacher who uses this text should augment these lists with additional questions adapted to the interests and background of his own student group.

The authors believe that effective teaching in any science not only should lead to the acquisition by the student of the basic facts, principles, and viewpoints of that science, but also should train the student in the use of that science in the interpretation of natural phenomena. Students should come to think of plant physiology not as a "subject" but as a useful tool which can be used in the explanation of plant behavior under natural or cultural conditions. Frequent exposure to properly selected problems and discussion questions will usually aid the student to use the facts and principles of a science as well as to learn them.

The manuscript has been read in its entirety by Dr. E. N. Transeau, Dr. H. C. Sampson, and Dr. R. O. Freeland of the Department of Botany of the Ohio State University, and by Dr. P. J. Kramer of the Department of Botany of Duke University. We are much indebted for constructive criticism to all these readers. Certain chapters have been critically read by Dr. W. G. France of the Department of Chemistry, the Ohio State University, Dr. R. C. Burrell of the Department of Agricultural Chemistry of the Ohio State University, Dr. W. H. Camp of the New York Botanical Garden, and Dr. T. Kerr of the United States Department of Agriculture. To these readers we are also grateful for a number of helpful suggestions. Thanks are also due to Dr. B. W. Wells of the Department of Botany of the College of Agriculture and Engineering of the University of North Carolina, for critical reading of certain chapters and for advice regarding the preparation of several of the figures.

With a few exceptions the figures are the work of Mrs. Celeste Taft, whose cooperation has aided considerably in their preparation. Those figures which have been redrawn from other sources as well as photographs which have kindly been furnished by several institutions and individuals have been properly credited in the text. Most of the anatomical drawings of leaves and stems and some others have been made from sections of living, undehydrated tissues. For this reason certain details of the tissue structure as depicted do not agree with frequently published figures of the same or similar tissues which are based on dehydrated and fixed tissue sections. In particular the cell walls of many tissues are shown to be thicker than is commonly indicated in similar

figures. We believe that the figures as published come closer to representing the actual anatomy of these tissues than would drawings of dehydrated and fixed sections.

The authors also wish to acknowledge that a number of the questions at the end of Chapter XXI, and a few in each of several other question lists were taken or adapted from lists prepared by Dr. O. F. Curtis of Cornell University.

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Columbus, Ohio
Raleigh, N. C.
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CHAPTER I

THE FIELD OF PLANT PHYSIOLOGY

In order to understand the mechanism of a plant in its entirety many separate phases of the dynamic activity of plants have been recognized and studied as individual processes. In his efforts to interpret these individual processes and their inter-relationships the plant physiologist is confronted with many problems: What is the mechanism by which water, gases, and solutes enter a plant from its environment? How do such substances pass out of a plant into its surroundings? How are foods and other complex organic compounds synthesized in the plant? How are they utilized in the development and maintenance of plants as living systems? What transformations of energy occur within a plant, and what exchanges of energy take place between a plant and its environment? How are water and solutes transported from one part of a plant to another? How are new tissues constructed? How is the development of one organ or tissue coordinated with the development of other organs or tissues? Why does a plant produce only vegetative organs at certain stages in its life cycle and reproductive organs only at other stages? How are individual plant processes and the development of a plant as a whole influenced by environmental conditions? All of these problems and many other related or subsidiary ones lie within the province of the branch of science which is known as plant physiology.

For convenience, the study of plant life is subdivided into various branches such as physiology, morphology, anatomy, ecology, pathology, genetics, etc. Such a classification is necessarily more or less arbitrary. Neither physiology nor any other phase of plant life can be singled out for study without some consideration of plants from other viewpoints. A particularly intimate interrelationship exists between the structures and processes of plants. Every physiological process is conditioned by the anatomical arrangement of the tissues, and by the size, configuration, and other structural features of the cells in which it occurs. Furthermore, the coordinated development of cells and tissues, *i.e.*, of the plant itself, is a complex of physiological processes. Thus the sciences of plant physiology and plant anatomy merge in the study of plant growth.

Just as different species of plants differ in outward configuration and internal anatomy, so do they also differ in physiology. The world of plants includes a great number of very diverse kinds of organisms that range in size from simple bacterial cells a few microns long to the enormous redwoods of our Pacific coast and mountain forests. Their difference in size, although striking enough, is not as fundamental as another distinction which exists between redwoods and bacteria. The redwoods and all other green plants are able to manufacture their own food, while the bacteria (with a very few exceptions) and all other non-green plants must obtain their nourishment from some outside source. The physiology of the green and the non-green plants is therefore basically unlike. This book is essentially a discussion of the physiology of the chlorophyllous (green) plants with the emphasis on the vascular green plants. The physiological processes of the bacteria and fungi have been considered only when they have a direct bearing on the physiology of the green plants.

With few exceptions all vascular green plants carry on the same fundamental physiological processes, but there are many differences in subsidiary processes. Most green plants, for example, synthesize starch, but many do not. Numerous similar variations occur in the kinds of metabolic products built up by various species of plants. Most of the physiological differences among the species of green plants, however, are quantitative rather than qualitative. All chlorophyllous plants carry on photosynthesis, but when different species are exposed to the same environmental conditions the rate at which the process takes place may differ greatly from species to species. A similar situation holds with respect to practically all of the other processes occurring in green plants. Even varieties of the same species often exhibit marked differences in physiological behavior when exposed to a given environmental complex. Some varieties of wheat, for example, are markedly cold resistant while others are not.

The Relation of Physiology to the Physical Sciences.—Formerly the opinion was almost universal that living organisms owe their distinctive properties to the possession of subtle and unknown forces which are peculiar to "living matter." At the present time such "vitalistic" theories find very few advocates. The contrary and now widely held assumption is that living organisms operate in accordance with the same physico-chemical principles that hold in the inanimate world. The complexity and elusiveness of living processes are not assumed to be due to intangible unknown varieties of energy, but to the interplay of recognizable physico-chemical forces in the complex organized system of the protoplasm.

Adoption of this latter point of view has led to a widespread use of the

tools of physics and chemistry in experimental work on plants, and to the interpretation of plant processes in terms of these two sciences. This has led to notable progress in our understanding of the physiology of plants and has permitted the analysis and expression of many physiological relations in quantitative terms.

A knowledge of certain fundamental principles of physics and chemistry is therefore essential to the understanding of physiological processes. For this reason several of the earlier and parts of some of the later chapters in this book are devoted to a brief exposition of those underlying principles of the physical sciences with which a student of plant physiology should be familiar.

The Relation of Plant Physiology to the Agricultural Sciences.— Green plants are not only the ultimate source of all food but supply the raw materials for many of our basic industries. With the rise of modern industrial civilization both the quantities and kinds of plant products which we utilize have increased rather than decreased. In addition to foods some of the more important raw products obtained from plants are wood, textile fibers, pulp, rubber, vegetable oils, gums and drugs. Even most of the so-called "synthetic products" of the chemist are not synthetic in the sense that they have been built up from simple inorganic compounds, but only insofar as they represent modifications of naturally occurring plant products.

An industrial civilization not only requires a wide variety of plant products but insists that these products meet certain standards of quality. The successful cultivation of plants has, therefore, become a highly skilled occupation, and the agricultural sciences are rapidly becoming a domain of the specialist. Success in controlling the activities of living plants can not be achieved without some understanding of the processes which occur within them, and of the effects of environmental conditions upon these processes. The problems of the forester, the fruit grower, the cotton planter, the floriculturist, the grain farmer, and of all others who cultivate plants differ in detail, yet they all have a fundamental similarity in requiring an application of the principles of plant physiology for their solution.

Fundamental investigations in plant physiology have contributed in many ways to improved methods of propagating, cultivating and harvesting economically important plants, and to methods of handling and storing many plant products. Furthermore, control of the fungous diseases and insect predators of plants often requires application of the principles of plant physiology. Much of the investigational work carried on by scientific agronomists, horticulturists, floriculturists, and foresters actually lies in the field of pure or applied plant physiology, although often it is not formally classed as such.

Plant Physiology as a Science.—The origin of man's first conscious interest in plants long antedates recorded history. Agriculture had already become a highly developed art thousands of years before any experimental study of plant processes began. Consequently, there had grown up a vast body of traditional plant lore which passed orally from father to son, generation after generation. This practical knowledge of plants developed over many centuries as a result of countless, mostly involuntary trial and error experiences and innumerable observations of plant behavior under all kinds of circumstances. Much of this mass of mingled facts and beliefs regarding plants is alive in the consciousness of the common man today. The closer he lives to the soil and the more familiar he is with traditional plant lore, the more it influences his thoughts and actions.

Many of these customarily accepted beliefs are essentially sound and most of them contain elements of truth. Others, however, are entirely erroneous, and not a few are tempered with superstitions, some of which have an unbroken lineage back to the days of witch-doctors and savagery. No reputable botanist has held for generations, for example, that plants obtain their food from the soil, yet this and many other fallacious beliefs are still widely entertained among the general population.

The value of practical information about plants should not be underrated, since its perpetuation in the mind of man permitted the development of agriculture to a high plane as a practical art before any widespread investigation of plants from a scientific point of view was undertaken. Nevertheless, traditional plant lore is not only often inadequate, but is riddled with misconceptions, and often suffers from points of view which are inherently stultifying to the acquisition of further knowledge.

The layman, for example, often personifies plants in an attempt to explain their behavior. Man has desires and foresight and it is often assumed, either consciously or tacitly, that plants are similarly endowed. To many, for example, the statement that "roots grow downwards in search of water," or that "stems grow upwards in order to reach the light" are accepted as adequate "explanations" of plant behavior. Man's knowledge that water and light are essential to plants is not evidence that plants are similarly aware of these facts. To assume that plants realize their needs and are able to act in conformity with their requirements is equivalent to crediting them with a high order of intelligence. Explanations of plant behavior are commonly encountered in which purposeful action on the part of plants is tacitly or deliberately implied, although there is no justification for the adoption of such a point of view.

Furthermore, the layman seldom pursues his quest for information about plants beyond the stage of observation, while the scientist frequently does.

Observation has suggested, for example, that light is necessary for the continued existence of plants. To one who is scientifically minded, either by instinct or training, the obvious next step is to test this postulate experimentally. If the suggested hypothesis is substantiated by experiment it is tentatively accepted as a theory. Theories such as those proposed in explanation of the processes or reactions of plants, together with the experimental results which are considered to support them, are usually published in a scientific journal or monograph and thus exposed to evaluation and further experimental testing by other scientists.

Continued experimentation may lead to substantiation, rejection, or modification of the theory as originally proposed. Modification would undoubtedly be the fate of the hypothesis used as an example, since sooner or later some investigator would find that non-green plants can thrive in the absence of light.

Experiments often raise more questions than they answer. New approaches to the problem under consideration as well as desirable new lines of inquiry are constantly opening up to the alert investigator. In this way experimentation leads to more experimentation, more facts accumulate, and more theories are proposed. Some of the suggested hypotheses are confirmed, others are rejected, and still others are modified. Most of them, sound or fallacious, in turn suggest further observation and experimentation. As a result of such endless and painstaking labors there is slowly built up that vast, complex, and ever-changing body of knowledge which we refer to as a science.

The system of subjecting all hypothetical explanations of natural phenomena to experimentation is the essence of the scientific method. It is the characteristic methodology of all sciences. Progressive modification of accepted concepts in the light of new experimental findings continually increases the soundness of scientific generalizations. Thus there are incorporated into any science theories and generalizations in various stages of acceptance. Some stand upon such a firm substructure of facts that they are accepted by all authorities in the field. Others, less securely supported by experimental results, are subscribed to by some but rejected by other workers. Finally, in any science there are always some theories which are so dubious that they find only a few advocates. Furthermore, some of the theories now widely held sooner or later will be discarded as a result of new findings or as a consequence of different interpretations of facts already known.

However, not all scientists are in agreement regarding the interpretation of the same sets of facts. Although this state of affairs is entirely consistent with the spirit of scientific research, it is frequently puzzling to students and laymen. Differences of opinion regarding the hypotheses which suitably explain scientific phenomena are most likely to arise when experimental data are

inadequate. Disagreements regarding the interpretation of experiments and observations are often inevitable steps in the clarification of scientific generalizations. Controversies usually focus attention upon gaps in our factual information. Frequently, therefore, they are stimulating to research, and often lead to a further enrichment of human knowledge.

Without the knowledge of plant processes which has been slowly accumulated through more than two centuries of observation, experimentation, and critical evaluation by numerous workers in all parts of the world, this book could never have been written. In spite of the patient labors of these many workers vast gaps still exist in our understanding of the physiology of plants—gaps which are reflected in the necessarily inadequate treatment of many topics in this book. The future of this science and all others lies in the hands of the front-line investigators who wage a continual struggle for the extension of human knowledge.

Suggested for Collateral Reading

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CHAPTER II

PROPERTIES OF SOLUTIONS

Water is the most abundant compound present in all physiologically active plant cells. The water which occurs in a liquid state in plant cells invariably contains other substances dissolved in it and usually also contains dispersed particles which are not in true solution. When the particles dispersed throughout the water are within a certain range of sizes the system of water plus particles falls into the category of *colloidal systems*. The complicated dynamics of living systems can be largely interpreted in terms of the physicochemical properties of solutions and colloidal systems, one component of which is water, although it should not be inferred that non-aqueous solutions and colloidal systems are entirely absent in living organisms.

Similarly liquid water never occurs in the pure state in the natural environment of living organisms. The water of streams, lakes, and oceans invariably contains various substances in solution and usually in the form of dispersed particles as well. This is likewise true of the soil water. Even raindrops, the products of natural distillation, contain gases which have dissolved in them from the atmosphere.

General Nature of Solutions.—Simple solutions are systems in which one component (the solute) is dispersed throughout the other (the solvent) in the form of molecules or molecules and ions. Theoretically the solvent may be a gas, a solid, or a liquid, but solutions in which the solvent is a liquid are by far the most important in living organisms. Except in extremely concentrated solutions the average distance between the solute particles is usually very great relative to their size. Naturally occurring solutions, whether in living organisms or their environment, usually contain a number of different solutes and are often exceedingly complex. Water is the commonest and most important of all solvents both in the inorganic world and in the realm of living organisms. The further discussion will be principally in terms of aqueous solutions.

Solutions of a Gas in a Liquid.—The water present in living organisms usually contains dissolved gases. Those most commonly present are carbon dioxide, oxygen, and nitrogen. Practically all the water in the environment

of organisms—in oceans, lakes, streams, soils, and raindrops—also contains these gases in solution, and sometimes others as well.

A given volume of water or any other liquid will hold only a limited quantity of gas in solution at a given temperature. When no more of a certain gas can be dissolved in a liquid it is said to be *saturated* with respect to that gas. Gases vary widely in their solubility in water, but in general fall into two groups: those which are sparingly soluble, and those which are highly soluble. When only a small fraction of a unit volume of a gas will dissolve in a unit volume of water the gas is classified in the sparingly soluble group. When from one to many unit volumes of a gas will dissolve in one unit volume of water it is classified in the highly soluble group.

Oxygen, hydrogen, and nitrogen are familiar examples of gases belonging to the first group, while carbon dioxide, ammonia, and hydrogen chloride are examples of the second. When gases are highly soluble in water it is usually evidence that a chemical reaction takes place between the gas and water. The reactions between water and carbon dioxide and water and ammonia are indicated in the following equations:

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$
$$NH_3 + H_2O \rightleftarrows NH_4OH$$

In a solution of such gases not only are molecules of the gas present, but also molecules of a compound formed by the reaction of the gas with water. The apparently great solubility of gases such as these is due to the formation of relatively soluble compounds by the reaction of the gas with the water. The solubilities of some common gases in water are shown in Table 1.

TABLE 1—SOLUBILITY OF SOME COMMON GASES IN WATER AT DIFFERENT TEMPERATURES WHEN THE PRESSURE OF THE GAS IS ONE ATMOSPHERE.

Gas	Volume of gas dissolved in one volume of water (reduced to standard conditions)		
	10° C.	20° C.	30° C.
Carbon Dioxide	1.194	0.878	0.665
Oxygen	0.0380	0.0310	0.0261
Nitrogen	0.0186	0.0154	0.0134
Hydrogen	0.0195	0.0182	0.0170

In general, as also shown in Table 1, an increase in temperature decreases the solubility of a gas in a liquid.

Increase in pressure of a gas above a liquid increases the solubility, *i.e.* the concentration of that gas in the liquid. For sparingly soluble gases the increase in solubility is directly proportional to the increase in pressure ("Law of Henry"). This principle also holds qualitatively, but not quantitatively, for the very soluble gases.

When a mixture of several gases is maintained over water, each dissolves independently and in accordance with the gaseous pressure ("partial pressure") which it exerts against the surface of the water ("Law of Dalton"). This principle holds strictly only for those gases which are slightly soluble in water. For example about one-fifth of the atmospheric pressure is due to the oxygen present. Assuming an atmospheric pressure of 760 mm. Hg (the value at standard conditions), the pressure due to the oxygen is equivalent to about 152 mm. Hg. The quantity of oxygen which dissolves in water exposed to the air is the same as that which would dissolve if oxygen only at a pressure of 152 mm. Hg occupied the space over the water.

Solutions of a Liquid in a Liquid.—In general solutions of a liquid in a liquid fall into two classes: those in which the liquids are freely miscible with each other in all proportions, and those in which each liquid reaches a definite point of saturation in the other. Alcohol and water, for example, mix with each other in all proportions; such a solution is an example of the first mentioned type. Many oily liquids also are miscible with each other in all proportions. A familiar example is the solution of lubricating oil in gasoline. In solutions of this kind the liquid present in excess is usually considered to be the solvent. In a 50 per cent solution of alcohol and water, either liquid could be considered the solvent, or either could be considered the solute.

Ether, chloroform, and many other liquids are sparingly soluble in water. After water and ether, for example, are shaken together in a flask, two distinct layers of liquid separate upon standing. The upper layer consists of the lighter ether, saturated with water, while the lower consists of water saturated with ether. In both layers, however, the concentration of solute present at saturation is small. In the upper layer ether is the solvent, water the solute; the converse is true of the lower layer.

Solutions of a Solid in a Liquid.—This is by far the most familiar type of solution and in many respects the most important. Substances in the solid state vary greatly in their solubility in water, ranging all the way from those which are virtually insoluble to those which are extremely soluble. There is usually a limit to the amount of any solute which can be dissolved in a given volume of water at a given temperature. When this limiting concentration is reached the solution is said to be *saturated,* following the same terminology used with solutions of gases and liquids in liquids. It is almost impossible to

prepare a true saturated solution of any solute unless some of it is also present in the solid state. Under certain conditions, and only when none of the undissolved solid is present, a *super-saturated* solution can be prepared; that is, a solution in which the concentration of the solute is greater than that in the saturated solution. If a fragment of the solid solute be added to such a solution the excess solute usually crystallizes out immediately, and the concentration of the solution decreases to that usually present at saturation.

Usually increase in temperature increases the solubility of a solid in a liquid but there are some exceptions to this principle. The solubility of common salt (NaCl) in water, for example, is only slightly influenced by temperature, while the solubility of many calcium compounds is decreased by an increase in the temperature of water.

Methods of Expressing the Composition of Solutions.—If a mol[1] of any soluble compound be dissolved in just enough water to make exactly one liter of solution the result is a *volume molar* solution. Since the volume of water changes with temperature it is usual to specify that the solution is to be made up to a liter volume at 20° C. Whenever the word *molar* is used without qualification this type of solution is meant. Similarly, if half the molar weight, or one-tenth the molar weight of a substance be dissolved in enough water to make exactly one liter of solution the result is a half molar (0.5 M) or tenth molar (0.1 M) solution, respectively, etc. Gram molar weights of all substances contain the same number of molecules. Estimates made by various methods show this number to be about 6.06×10^{23} molecules. Hence one liter of any volume molar solution will contain this number of solute molecules, one milliliter (0.001 liter) will contain one thousandth of this number, and so on. Equal volumes of all solutions of the same volume molarity contain the same number of solute molecules but different numbers of solvent molecules. If a given volume of a volume molar solution be diluted with an equal volume of water, the result is a 0.5 M solution; if a given volume be diluted with nine volumes of water the result is a 0.1 M solution, etc. Therefore a volume molar solution of any strength may be diluted with water, and the resulting more dilute solution will have a volume molarity in proportion as it has been diluted.

If a mol of any soluble substance be completely dissolved in 1000 g. of water the result is a *weight molar* solution. Such solutions are often called *molal* solutions. They are used principally in experimental work upon various osmotic phenonema. The addition of a mol of most solids to a liter of water will increase the volume of the resulting solution to more than one liter. This increase in volume is called the *solution volume* of the solute. The solution

[1] A mol is the molecular weight of a compound in grams.

volume of many substances is very small, and for a few it is even negative, *i.e.* there is a shrinkage in volume when the solute is added to the solvent. On the other hand the solution volume of some compounds, especially the sugars, is considerable. When a mol of sucrose is added to 1000 g. of water the resulting solution will have a volume of 1207 cc. at 0° C. Hence the solution volume of sucrose is 207 cc. The solution volume of a mol of sodium chloride, on the other hand, is only about 18 cc. Since every solute has a different solution volume, it follows that equal volumes of weight molar solutions do not contain the same number of either solvent or solute molecules. Neither will the dilution of a given volume of a weight molar solution with an equal volume of water result in a 0.5 molal solution. In other words the concentration of a weight molar solution does not change in proportion to the amount by which it has been diluted. This is a fact which is sometimes overlooked in experimental work.

In physiological work it is often convenient to make up solutions on a percentage basis. Such solutions are made up either on the basis of percentage by weight, or percentage by volume. Solutions of solids in water or other solvents are made up on a weight percentage basis. A 10 per cent sodium chloride solution, for example, is made by dissolving 10 g. of sodium chloride in 90 g. of water. Solutions of liquids in water or other solvents can also be prepared on a weight percentage basis. It is simpler, however, to make up such solutions on a volume percentage basis. On this basis a 10 per cent solution of alcohol is prepared by adding 10 cc. of alcohol to 90 cc. of water. The percentage system of solutions has no direct relation to either the volume molar or weight molar systems.

Electrolytes and Non-Electrolytes.—Some aqueous solutions readily conduct an electric current; others do not. The former are called *electrolytes;* the latter *non-electrolytes.*[2] The solutions of all acids, bases, and salts are electrolytes. Solutions of most organic compounds such as the sugars, alcohols, ketones, and ethers are non-electrolytes.

Passage of an electric current through an electrolyte results in its decomposition. This process is called *electrolysis.* If hydrochloric acid is the electrolyte, for example, hydrogen gas will be liberated at the negative pole (*cathode*) and chlorine gas will be liberated at the positive pole (*anode*). If electrolysis is continued long enough eventually all of the hydrochloric acid present in the system will be decomposed into hydrogen gas and chlorine gas.

[2] Strictly speaking the term electrolyte refers only to a *solution* of an ionized substance, but it is also often applied to any *compound* which, when dissolved in water, produces ions. A similar dual usage of the term non-electrolyte also prevails.

The occurrence of electrolysis, as well as the unusually large effects of electrolytes on the osmotic pressures of solutions (Chap. VIII), have led to an explanation of the behavior of electrolytes in terms of the theory of electrolytic dissociation. This theory was first advanced by the Swedish chemist Arrhenius who introduced it in essentially its present form in 1887. According to the Arrhenius theory when an electrolyte is dissolved in water some of the molecules *dissociate* into two kinds of particles, one positively charged, the other negatively charged. Each of these particles is called an *ion*. Ions are supposed to be present in a solution whether any electrolysis occurs or not. Two, three, four or even more ions may be formed from a single molecule. The conduction of an electrical current by an electrolyte is due to the presence of these ions. The dissociation of several typical electrolytes is indicated by the following equations:

$$NaCl \rightleftarrows Na^+ + Cl^-$$

$$CaCl_2 \rightleftarrows Ca^{++} + Cl^- + Cl^-$$

$$Na_2SO_4 \rightleftarrows Na^+ + Na^+ + SO_4^{--}$$

The positively charged ions, which in electrolysis migrate towards the cathode, are called *cations;* the negatively charged ions which migrate toward the anode are called *anions*. A cation may carry one, two, three, or even four positive charges; an anion may carry from one to several negative charges. When an electrolyte dissociates the number of positive charges carried on the cations is always equal to the number of negative charges carried by the anions.

The Arrhenius theory does not assume complete ionization of all of the solute molecules in an electrolyte but that the molecules are continuously dissociating into ions, while free ions in the solution are continuously reuniting and forming molecules, both processes proceeding at an equal rate whenever an equilibrium condition prevails. An equilibrium of this sort, which is maintained by two opposing processes proceeding at equal rates is termed a *dynamic equilibrium*. Electrolytes vary greatly in degree of dissociation (Table 2). Those in which a large proportion of the molecules are maintained in a dissociated condition are termed "strong" electrolytes; those of which the contrary is true, "weak" electrolytes. "Strong" electrolyte solutions conduct electric currents better than "weak" electrolyte solutions of equal concentration. In general the more dilute a given electrolyte solution the larger the proportion of dissociated molecules present. In extremely dilute solutions ionization is practically complete. Increase in temperature reduces the dissociation of an electrolyte.

TABLE 2—DEGREE OF DISSOCIATION OF NORMAL SOLUTIONS OF SOME COMMON ELECTROLYTES

Electrolyte	Degree of Dissociation	Electrolyte	Degree of Dissociation
HNO_3	82 per cent	$Ba(OH)_2$	69 per cent
HCl	78	KCl	74
H_2SO_4	51	$BaCl_2$	57
KOH	77	K_2SO_4	24
NaOH	73	$CuSO_4$	22

It is probable that many of the so-called non-electrolytes also dissociate very slightly in solution, but the degree of dissociation of such compounds is so small that it can be detected only by very refined methods, if at all. Even water, as the subsequent discussion will show, dissociates slightly, producing hydrogen and hydroxyl ions.[3]

Acids, Bases, and Salts.—An acid may be defined as a substance which produces hydrogen ions (H^+) when dissolved in water. The characteristic properties of acids are due to the hydrogen ions produced. The most common inorganic acids are hydrochloric (HCl), nitric (HNO_3), and sulfuric (H_2SO_4). In living organisms a large group of more complex, but much weaker acids, known as organic acids, play important roles. The "strength" of an acid depends upon its degree of ionization; the greater the proportion of hydrogen ions an acid produces in solution at a given concentration, the "stronger" it is.

A base may be defined as a substance which produces hydroxyl ions (OH^-) when dissolved in water. The characteristic properties of bases are due to the hydroxyl ions produced. Some of the commonest bases are sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), and potassium hydroxide (KOH). The "strength" of a base, like that of an acid, depends upon its degree of ionization. The greater the proportion of hydroxyl ions a base produces in solution at a given concentration, the "stronger" it is.

[3] The theory of electrolytic dissociation accounts much more satisfactorily for the behavior of weak electrolytes than of strong electrolytes. For this and other reasons Debye and Hückel in 1923, and other modern investigators, have postulated that strong electrolytes, at least, are completely dissociated. The fact that they behave as if only partly dissociated is accounted for in terms of interionic attractions. These attractions are supposed to exert what may be roughly described as a "braking effect" upon the movement of the individual ions. This prevents them from operating at their maximum effectiveness in conducting an electric current or in other phenomena depending upon ionic mobilities. The greater the concentration of an electrolyte, the closer together are the ions, and, according to this view, the less their apparent dissociation.

A salt may be defined as a compound which has been produced by the union of an anion or anions of an acid with the cation or cations of a base. Salts are produced when an acid and a base are brought together in a solution, as a result of a chemical union between the hydrogen ion(s) of the acid and the hydroxyl ion(s) of the base, forming water. This reaction is called *neutralization.*

Following are several examples:

$$HCl + NaOH \rightleftarrows NaCl + H_2O$$
$$H_2SO_4 + 2\,KOH \rightleftarrows K_2SO_4 + 2\,H_2O$$
$$2\,HCl + Ca(OH)_2 \rightleftarrows CaCl_2 + 2\,H_2O$$

Since water is practically undissociated, neutralization reactions go rapidly to completion. The reverse reaction, indicated in the above equations by the arrows pointing to the left, is called *hydrolysis.* Under certain conditions hydrolytic reactions may proceed at a rapid rate, although in the examples presented above the speed of the reaction towards the left (hydrolysis) is negligible compared with the speed of the reaction towards the right (neutralization).

Normal Solutions.—The concentrations of acids and bases are most commonly expressed in terms of normal solutions. A *normal solution* of an electrolyte contains in a dissolved state per liter of solution at 20° C. a weight of the compound in grams equal to its molar weight divided by its hydrogen equivalent. The *hydrogen equivalent* of a compound is defined as the number of replaceable hydrogen atoms in one molecule, or the number of atoms of hydrogen with which one molecule could react. Thus a normal solution of an acid contains 1.008 g. of replaceable hydrogen per liter; a normal solution of a base 17.008 g. of replaceable hydroxyl per liter. By this system the concentration of any acid, base, or salt can be designated as 0.1 N, 0.5 N, 2 N, etc., as the case may be.

The normality of an acid solution is a measure of its *total acidity, i. e.* of its concentration in terms of replaceable hydrogen ions. Similarly the normality of a base solution is a measure of its *total basicity.* Since 1.008 g. of replaceable hydrogen represents the same number of ions as 17.008 g. of replaceable hydroxyl (why?) it is evident that equal volumes of acid and base solutions of equal normality will exactly neutralize each other.

Hydrogen Ion Concentration.—The effects of acids upon chemical reactions and upon physico-chemical conditions generally in both inorganic systems and in living organisms are due principally to the hydrogen ions which they produce when in solution. Some of the most fundamental of physiological

phenomena are markedly influenced by the concentration of hydrogen ions in the medium in which they occur. For many purposes therefore it is more important to have some sort of a measuring stick of the concentration of hydrogen ions present in a solution than of the total acidity of the solution.

Total acidity, as already noted, is customarily expressed in terms of normality. It is also entirely possible to use the normal system for expressing the concentration of hydrogen ions. In a normal solution of hydrochloric acid, for example, about 78 per cent of the molecules are dissociated. The term normal as used in the preceding sentence refers to total acidity, that is, to all the ionizable hydrogen whether actually present as ions or combined with anions in the form of molecules. In terms of the hydrogen *ions* present, however, such a solution is only 0.78 N. The term normal as used in this latter sense refers only to the *ionized* hydrogen. We may speak therefore of a normal solution of hydrogen ions as well as of a normal solution of an acid.

Since no acid is ever completely dissociated, a normal solution of any acid will always be less than normal when its concentration is expressed in terms of the hydrogen ions present. In order to prepare a normal solution of hydrogen ions it is necessary to make up a solution which is more than normal in terms of total acidity. Such a solution must be of the precise strength and degree of dissociation that exactly 1.008 g. of the ionizable hydrogen present are actually in the dissociated form—as ions—per liter of the solution.

Although hydrogen ion concentration can be readily expressed in terms of normalities, in actual practice this system is not generally used because it is apt to prove cumbersome, especially when it is necessary to refer to the very small concentrations of hydrogen ions usually dealt with in biological problems. The hydrogen ion concentration of a solution is now quite generally defined in terms of its pH value. The pH value bears a simple mathematical relation to the hydrogen ion concentration of a solution in terms of its normality. Because of the practically universal acceptance of this system it is necessary to understand the significance of the term pH and its relation to hydrogen ion concentration expressed in terms of normality.

The relation between pH and hydrogen ion concentration in terms of normality is a logarithmic one (Table 3). The pH of a normal solution of hydrogen ions is 0, of a 0.1 N solution 1, of a 0.01 N solution 2, etc. Zero is the logarithm of 1, 1 is the logarithm of 10, 2 is the logarithm of 100, etc. The pH value for any solution is the negative of the logarithm of the hydrogen ion concentration in terms of normality. It may also be defined as the logarithm of the number of liters of solution that contains one gram atomic weight of hydrogen ions.

All aqueous solutions as well as pure water contain hydrogen ions in some

concentration. Corresponding to the concentration of hydrogen ions is a certain definite concentration of hydroxyl ions. This concentration of hydroxyl ions is indicated in Table 3 as pOH. It can be shown by the principle of mass action that the mathematical product of the concentration of hydroxyl ions and the concentration of hydrogen ions in a solution is a constant. This may be expressed as follows:

$$(H^+) \times (OH^-) = K. \qquad K = 10^{-14} \text{ at } 22^\circ \text{ C.}$$

Hence as the pH of a solution is increased the pOH decreases and *vice versa.* For example, if the pH increases from 5 to 6 the pOH decreases from 9 to 8.

Furthermore, only at pH 7 can the concentration of hydrogen ions equal the concentration of hydroxyl ions. This is therefore the neutral point on the pH scale and corresponds to the dissociation of pure water. This pH value represents a dissociation of only one water molecule in approximately every 555,000,000.

Values below 7 on the pH scale represent the acid range, those above 7 the alkaline range of the scale. An "acid" solution is one with a larger concentration of hydrogen ions than hydroxyl ions, while in an "alkaline" solution the reverse is true. The lower the pH value the greater the hydrogen ion concentration of a solution. A pH value of 5 represents ten times the hydrogen ion concentration of a solution with a pH of 6 and one hundred times the hydrogen ion concentration of one with a pH value of 7, etc. This is due to the fact, previously emphasized, that the numbers on the pH scale are related to each other as logarithms and not as ordinary arithmetic numbers.

The hydroxyl ion concentration of a solution could be expressed in pOH units instead of in pH units. For alkaline solutions especially this would seem to be a logical practice. But because of the definite mathematical relationship between the pH and pOH the pH value alone also defines the pOH value. Hence both the acidity of a solution in terms of H^+ ions, and its alkalinity in terms of OH^- ions may be, and customarily are, expressed in terms of pH units.

Table 3 shows only the pH values corresponding to the range between a normal solution in terms of hydrogen ions and a normal solution in terms of hydroxyl ions. It is possible for aqueous solutions to exist which have a pH value of less than 0 (*i.e.* a minus pH value) or more than 14. A 5 N solution of hydrochloric acid, for example, would have a pH value of a little less than 0; correspondingly a 5 N solution of sodium hydroxide would have a pH value a little greater than 14. In actual experience, however, solutions of such extreme concentrations are seldom encountered.

TABLE 3—THE RELATION OF pH TO HYDROGEN-ION CONCENTRATION EXPRESSED IN TERMS OF NORMALITY

H ion concentration in terms of normality	1	.1	.01	.001	.0001	.00001	.000001	.0000001	.00000001	.000000001	.0000000001	.00000000001	.000000000001	.0000000000001	.00000000000001
	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-13}	10^{-14}
pH	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
pOH	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
	10^{-14}	10^{-13}	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{0}
OH ion concentration in terms of normality	.00000000000001	.0000000000001	.000000000001	.00000000001	.0000000001	.000000001	.00000001	.0000001	.000001	.00001	.0001	.001	.01	.1	1
	Acid Range								Alkaline Range						

Table 4 gives the pH values for solutions of some common acids and bases.

TABLE 4—pH VALUES OF SOME COMMON ACIDS AND BASES

Acid or Base	Normality	pH
HCl	1.0	0.10
HCl	0.1	1.071
CH_3COOH	1.0	2.366
CH_3COOH	0.1	2.866
NaOH	1.0	14.05
NaOH	0.1	13.07
NH_4OH	1.0	11.77
NH_4OH	0.1	11.27

Buffer Action.—Suppose that 1 cc. of a normal hydrochloric acid solution be added to 10 cc. of a normal solution of sodium chloride. The initial pH value of this latter solution will not be sensibly different from that of the water with which it is made.[4] It will be found that the pH of the solution will drop abruptly to a value of about one. If, on the other hand, a

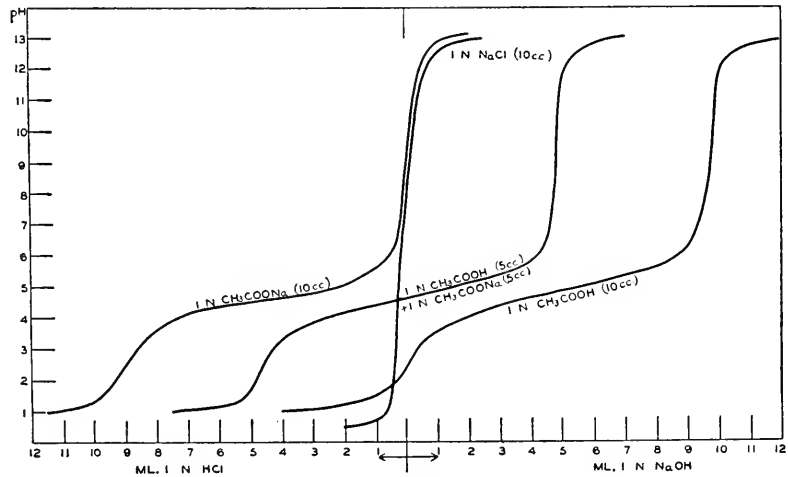

FIG. 1. Curves illustrating buffer action.

normal hydrochloric acid solution be added, 1 cc. at a time, to 10 cc. of a normal solution of sodium acetate it will be found that change in the pH of this solution takes place much more slowly. These facts, as well as the others discussed in this section, are illustrated graphically in Fig. 1. It is evident

[4] The pH of distilled water when in equilibrium with the carbon dioxide of the atmosphere is about 5.7.

that the sodium acetate solution retards in some way changes in pH value upon the addition of an acid while a solution of sodium chloride does not.

Solutions of such compounds as sodium acetate which are relatively resistant to changes in pH due to the addition or loss of hydrogen or hydroxyl ions are known as *buffer solutions,* and this property of solutions is called *buffer action.* Solutions such as sodium chloride which show no buffer effect are called *unbuffered* solutions.

If 1 cc. of a normal sodium hydroxide solution be added to 10 cc. of a normal solution of sodium chloride, a marked increase in pH will occur. If 1 cc. of the same solution be added to 10 cc. of a normal solution of sodium acetate, its pH will also increase markedly. In other words the sodium acetate solution is buffered against the addition of an acid, but not against the addition of a base. If, on the other hand, 10 cc. of a normal solution of acetic acid be substituted for the sodium acetate it will be found that this solution is strongly buffered against the introduction of hydroxyl ions into the solution. A mixture of equal volumes of molar sodium acetate solution and molar acetic acid solution will exhibit buffer action against both acids and bases over a considerable range of the pH scale.

Two important points regarding buffer solutions have been brought out by the foregoing discussion. No one solute as a rule will act as a buffer against both acids and bases. Furthermore no buffer solution will exert a buffer effect over the entire range of the pH scale. Different buffer solutions vary greatly in their effectiveness in maintaining pH stability. Some are strongly buffered; others weakly. Some are strongly buffered against acids and weakly buffered against bases; of others the converse is true. The commonest types of buffer systems are those composed of a weak acid plus one of its salts. The sodium acetate-acetic acid buffer system already described is such a system. Practically all of the buffer solutions of importance in living organisms belong to this group.

Buffer action consists essentially in the tying up of free hydrogen or hydroxyl ions nearly as rapidly as they are introduced into the solution in the formation of compounds which are only slightly dissociated. The ensuing change of pH is therefore relatively small in proportion to the volume of acid or base added. As an illustration let us consider once more a solution consisting of both sodium acetate and acetic acid dissolved in water.

These two compounds dissociate as follows:

$$CH_3COONa \rightleftarrows CH_3COO^- + Na^+$$

$$CH_3COOH \rightleftarrows CH_3COO^- + H^+$$

The sodium acetate is strongly dissociated, but the acetic acid will dissociate only slightly, being a weak acid.

Suppose now that a little HCl be added to this solution. This is equivalent to adding H^+ and Cl^- ions and HCl molecules; the latter, however, will dissociate, forming additional ions as rapidly as the H^+ and Cl^- ions already present are bound up in chemical combination. H^+ and CH_3COO^- ions cannot exist side by side in the same solution in appreciable concentrations since CH_3COOH is a poorly dissociated compound. Hence the added H^+ ions are almost all tied up in the formation of CH_3COOH. The Cl^- ions form NaCl with the Na^+ ions which dissociates in the usual way. The result is that there is only a slight increase in the concentration of hydrogen ions in the solution, and hence only a very slight reduction in pH value.

Suppose now that instead of HCl, a little NaOH be added to this solution. This is equivalent to adding Na^+ and OH^- ions and NaOH molecules; the latter, however, will produce additional ions by dissociating as rapidly as the Na^+ and OH^- ions already present are tied up in chemical combination. But OH^- and H^+ ions cannot exist side by side in the same solution in appreciable concentrations since H_2O is only slightly dissociated. Most of the added OH^- ions therefore combine with the H^+ ions produced by the CH_3COOH and form H_2O. More of the CH_3COOH hydrolyzes producing more H^+ ions, which in turn unite with more of the OH^- ions. This continues until practically all of the added OH^- ions are tied up. The Na^+ ions form CH_3COONa with the CH_3COO^- ions which dissociates in the usual way. The final result is that there is only a very slight decrease in the concentration of H^+ ions in the system, and hence only a very slight increase in its pH value.

Any mechanism which will act in such a way as to remove hydrogen or hydroxyl ions from a solution may operate as a buffer system. Other types of buffering are known but they are relatively of much less importance in living organisms than the type of chemical mechanism which has just been considered.

Hydration of Solutes.—Molecules of water adhere to the particles of many solutes. Water thus associated with the particles of a solute is called *water of hydration.* For example, each molecule of dissolved sucrose apparently has six molecules of water associated with it. This means that if a mol of sucrose (342.2 g.) is dissolved in water there will be bound to the sucrose molecules a total of six mols of water (108.096 g.). Ions also are hydrated. Different species of ions appear to have different numbers of water molecules associated with them. Although exact values have been assigned by several experimenters for the number of molecules of water of hydration

for certain ions these values are open to question as the results of different investigations do not agree. It seems certain, however, that both anions and cations are hydrated, and that for some kinds of ions, at least, as many as a hundred or more molecules of water of hydration may be associated with a single ion.

Discussion Questions

1. Describe the appearance of a solution of sucrose in water assuming you were small enough to stand between the water molecules. Do the same for a solution of sodium chloride. What changes would be observed upon a rise in temperature?
2. How would you prepare a volume molar solution of glucose ($C_6H_{12}O_6$), NaCl, $CaCl_2 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$? Weight molar solutions of the same compounds?
3. Given a volume molar solution, how would you prepare 125 cc. of a 0.75 volume molar solution? Given a 0.5 volume molar solution, how would you prepare 50 cc. of a 0.225 volume molar solution?
4. What is the weight molar concentration of a 20 per cent sucrose solution?
5. When a weight molar solution of sucrose is prepared by adding 342.2 g. of sucrose to 1 liter of water the volume of the resulting solution is 1207 cc. at 0° C. What is its volume molar concentration?
6. How much water must be added to the 1207 cc. of weight molar solution (question 5) in order to convert it into a 0.5 weight molar solution?
7. How much 0.2 N NaOH would be required to neutralize 100 cc. 1 N HCl?
8. If 15 cc. of KOH solution requires 17.5 cc. of 1 N HCl solution to neutralize it, what is the normality of the KOH solution?
9. If an electrolyte which produces two ions is 50 per cent dissociated what will be the per cent of dissolved particles (ions plus molecules) in the solution in terms of the number of particles which would be present if no dissociation occurred? If the electrolyte were 75 per cent dissociated? Answer the same questions for an electrolyte which produces three ions.
10. If the pH of a molar solution of hydrochloric acid is 0.1, and the pH of a molar solution of acetic acid 2.37, what would the total acidity of each of these two solutions be?
11. How much greater is the concentration of hydrogen ions in a solution of pH 4 than one of pH 7? One of pH 3 than of pH 9?
12. How much greater is the concentration of hydroxyl ions in a solution of pH 13 than one of pH 10? One of pH 8 than one of pH 5?
13. Why will an increase in the CO_2 content of water result in an increase in its H-ion concentration?
14. Why will the addition of a base to water decrease the concentration of hydrogen ions present?
15. In two determinations of the pH of a solution an investigator obtained values of 6.0 and 6.4. He concluded that an average value for the pH of the solution was 6.2. Evaluate.

Suggested for Collateral Reading

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CHAPTER III

INTERFACIAL PHENOMENA

There are a number of familiar observations which indicate that the surface layer of water possesses certain distinctive properties which are not exhibited within the body of the liquid. For example, if a perfectly dry, clean, steel needle be carefully laid on the surface of some still water it will float, in spite of the fact that steel is heavier than water (Fig. 2). Careful observation shows that the water surface beneath the needle is depressed, forming a tiny liquid cradle, within which the needle is supported. This phenomenon is clearly due to the properties of the surface film of the water, because if the needle is laid on the water at a slight angle, so that this film is punctured, it will immediately sink to the bottom of the vessel. A number

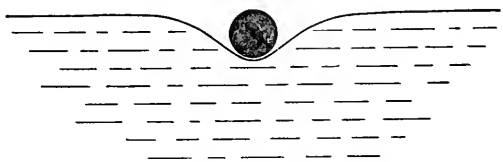

FIG. 2. Needle floating on water.

of other familiar phenomena are possible principally because of the distinctive properties of the surface film of water. The ability of many kinds of insects to "walk" on water, the formation of drops by water under certain conditions, the rise of water in capillary tubes, soil, blotting paper, etc., the upward bulging of the water surface in a vessel when it is filled to the very last drop, and many other aspects of the behavior of water, are due largely or entirely to the properties of its surface layer. The surface layers of other liquids also exhibit distinctive properties, but in no common liquid except mercury are such properties as well marked as in water.

Surface Tension.—Every molecule within the body of a liquid, although in rapid motion as a result of its kinetic energy, attracts and is attracted by other molecules. The attractive forces which any molecule exerts upon its neighbors may be considered as acting along lines of force which radiate from

it in all directions. The pull exerted by any molecule upon surrounding molecules, however, diminishes very rapidly with increasing distance from that particular molecule, reaching a maximum at distances not exceeding one or two molecular diameters. Liquids possess a definite volume and distinct boundaries because the molecules are close enough together to attract each other with forces of considerable magnitude. The important property of *cohesion* in liquids is due to these forces. The magnitude of the cohesive force between the molecules is greater in water than in most liquids. The molecules of a solid are so closely bound together by intermolecular attractions that it has lost ability to change its form, and possesses not only definite, but fixed boundaries. The molecules of both liquids and solids are kinetically active, but their movements are limited more or less rigidly by cohesive forces between the molecules. This limitation upon the freedom of movement of molecules is much greater in solids than in liquids. In gases the distance between the molecules is relatively so great that the intermolecular attractive forces are of negligible magnitude.

Molecules at the surface of a liquid or a solid are affected differently by the cohesive forces between molecules than those within the interior. The further more detailed discussion of this point will be in terms of water. In a vessel of water each of the individual molecules in the body of the liquid is attracted in all directions by the cohesive forces exerted upon it by the surrounding molecules. Any individual molecule is influenced in this way by a large number of its neighboring molecules. As long as any given water molecule is well within the body of the liquid the attractive forces which it sustains will balance each other, so that the molecule is subjected to an equal pull in all directions. At the surface, however, the molecules are not surrounded on all sides by other water molecules. Every molecule in the surface layer is pulled strongly toward the interior by the water molecules beneath it. It is for this reason that drops of water falling freely through space acquire approximately a spherical shape. Since there are no outwardly directed attractions which balance these internally directed forces the surface layer of molecules in any liquid is constantly under a tension. These conditions are illustrated diagrammatically in Fig. 3.

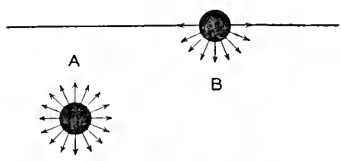

FIG. 3. A diagrammatic representation of the direction of the forces of cohesion acting upon a water molecule: (*A*) in the body of the liquid, (*B*) at its surface.

The strained condition of the surface layer of liquid molecules at liquid-gas boundaries is known as *surface tension*. Solid surfaces also manifest surface tensions, but gases, having enormous intermolecular distances and no

surface boundaries, do not. In addition to the unbalanced molecular attractions which the molecules in the surface layer of a liquid sustain, such molecules are usually definitely oriented. This results in a greater concentration of molecules in the surface layer than in the body of the liquid, and contributes toward maintenance of rigidity of the surface film. Surface tension values are generally stated in terms of dynes per centimeter, that is, as the force in dynes which is exerted by the liquid along a line 1 cm. in length. The surface tension values of some common liquids are given in Table 5.

TABLE 5—SURFACE TENSIONS OF SOME COMMON LIQUIDS AT 20° C.

Acetic Acid	27.6 dynes/cm.
Benzene	28.9
Chloroform	27.1
Ethyl alcohol	22.3
Ethyl ether	17.0
Mercury	465.0
Methyl alcohol	22.6
Water	72.8

Factors Affecting Surface Tension.—1. *Temperature.*—Since the phenomenon of surface tension is one manifestation of the cohesive forces existing among molecules, the greater these cohesive forces, the greater the surface tension. Temperature is a measure of the kinetic energy of the molecules, hence an increase in temperature means an increase in their kinetic energy and *vice versa.* As the kinetic activity of water molecules increases, the effectiveness of the cohesive forces existing between them decreases. Hence as the temperature of water increases, its surface tension decreases. The decrease in surface tension of any liquid is, in fact, directly proportional to increase in temperature, except when a liquid approaches its critical temperature. The variation in the surface tension of water within the temperature range at which most physiological processes occur is not sufficient, however, to be of any very great significance.

2. *Solutes.*—As shown in Table 6, the surface tension of a liquid is influenced by the presence of the molecules or ions of a solute. Some solutes increase the surface tension of water; others decrease it. If alcohol, for example, be added to water the surface tension of the resulting solution will be less than that of pure water. The attraction between an alcohol molecule and a water molecule is less than that between two water molecules. Since the alcohol molecules are distributed among the water molecules, the mean cohesive force in the solution is less than that in pure water. The reduction

in surface tension occasioned by the addition of alcohol to the water is one aspect of the reduction of the cohesive forces between the molecules in the solution. Most organic compounds have a similar and often very marked effect on the surface tension of water when dissolved in it. However a few such as sucrose have a slight increasing effect on surface tension.

Most inorganic salts when dissolved in water increase its surface tension, as shown in Table 6. The presence of such solutes increases the cohesion within the liquid by attracting the water molecules more strongly than the water molecules attract each other. The mean cohesive force of the molecules in the solution is thus increased. The result of this effect is a greater surface tension in the solution than in pure water. The magnitude of the effect of inorganic salts upon surface tension is never very great, however.[1]

TABLE 6—THE EFFECTS OF VARIOUS SOLUTES UPON THE SURFACE TENSION OF WATER

Solute	Temperature ° C.	Surface tension of water at indicated temperature	Surface tension of weight-molar solutions of indicated solute at indicated temperature
Acetic acid	30	71.2	59
Acetone	25	72.0	55
Ethyl alcohol	30	71.2	56
Methyl alcohol	30	71.2	64
Sucrose	25	72.0	73
Sodium chloride	20	72.8	74.4
Calcium chloride	25	72.0	75.2
Magnesium chloride	20	72.8	75.8
Sodium sulfate	20	72.8	75.5

As a rule, increasing the concentration of a solute in water increases the magnitude of its effect, whether positive or negative, upon surface tension. The influence of solutes upon surface tension is not proportional to their concentration, however, as low concentrations of any solute produce relatively greater effects upon surface tension than high concentrations.

Interfacial Tension.—The condition of tension or strain in a limiting layer of molecules is not confined to boundaries between liquids and gases. At a boundary between two immiscible liquids or between a solid and a liquid each of the abutting layers of molecules is subject to similar tensions. Boun-

[1] In general compounds which decrease the surface tension of water are of the type known as "non-polar," while those which increase it are of the type known as "polar" (Chap. X).

daries of this sort are usually referred to as *interfaces*, and the tensions developed at interfaces are called *interfacial tensions*. The term surface tension is usually restricted to tensions developed at the surface of a liquid when in contact with a gas, *i.e.* at the "interface" between a liquid and a gas. Surface tension is therefore merely one variety of interfacial tension. The interfacial tensions of a number of pure liquids as measured against a water surface are given in Table 7.

TABLE 7—INTERFACIAL TENSIONS OF LIQUIDS AGAINST WATER AT 30° C.

Liquid	Interfacial tension in dynes per cm.
Benzene	32.5
Carbon tetrachloride	42.7
Chloroform	31.4
Ethyl ether	11.1
Toluene	34.6
Turpentine	23.0

The magnitude of interfacial tensions is influenced by the factors of temperature and solutes in the same general way as surface tensions.

Adsorption.—The molecules of most organic compounds, as we have already seen, have a lesser attraction for water molecules than the molecules of water have for each other. This relatively greater attraction between water molecules than between the molecules of water and those of such "non-polar" solutes results in the displacement into the surface or interfacial layers of a disproportionately large number of the solute molecules. Hence the concentration of most organic solutes is greater in the surface or interfacial layers than in the body of the liquid. The concentrating of solute molecules in the surface or interface of a liquid is called *positive adsorption*.

The molecules of inorganic salts, on the other hand, are more strongly attracted to water molecules than the water molecules are attracted to each other. Because of this stronger attraction of the water molecules for such "polar" solutes relatively more solute molecules are pulled from the surface films into the body of the liquid than water molecules. Molecules of "polar" solutes, therefore, generally occur in greater concentration in the body of the liquid than in the surface layers. This phenomenon is called *negative adsorption*. When negative adsorption occurs the *solvent* molecules become relatively more concentrated in the surface layer than in the body of the liquid. For reasons which will become clear later, negative adsorption rarely occurs at interfaces.

The difference in concentration between the surface layer and interior of a liquid are in general much less in negative adsorption than in positive adsorption.

Adsorbed molecules are not static, but exhibit a continuous although reduced kinetic activity. Molecules adsorbed at surfaces or interfaces are in dynamic equilibrium with the molecules of the same species in the body of the liquid. An adsorption equilibrium is attained when the number of adsorbed molecules passing out of the interface in a unit of time is equal to the number passing into the interface.

When adsorption occurs from a liquid containing many solutes, as is true of most biological liquids, all solutes are adsorbed to a greater or lesser degree, depending upon their specific properties in relation to the adsorbing surface. In general, however, under such conditions no one solute will be adsorbed as completely as if it alone were present. If a substance is introduced into an aqueous solution which will lower the surface tension of water more than another substance, already adsorbed, the first compound will largely displace the second compound in the interfaces of that solution.

Interfacial Adsorption.—Adsorption occurs at all types of interfaces. Many solids adsorb gas molecules very powerfully at solid-air interfaces. Solutes may be adsorbed at interfaces between immiscible liquids, and at interfaces between liquids and solids, as well as at liquid-gas interfaces ("surfaces"). Water molecules are strongly adsorbed at many solid-liquid interfaces. Adsorption is, therefore, a phenomenon of a very general occurrence.

Adsorption at liquid-gas interfaces involves only the forces of attraction between the solvent and solute molecules, since the molecules of a gas are too far apart to have any appreciable attraction for the solute molecules. Similarly adsorption at solid-gas interfaces involves only the forces of attraction between the molecules of the solid and those of the adsorbed gas. At liquid-liquid, or solid-liquid interfaces, however, adsorbed molecules are subject to the attraction of the molecules of the two abutting substances at the interface. At an interface between carbon particles and water, molecules are attracted by both the carbon and the water. If the carbon exerts a greater attraction for the solute than the water—as is usually the case—a greater accumulation of solute molecules will occur in the interface, than if the opposite is true. The concentration of adsorbed molecules which will be attained at any solid-liquid or liquid-liquid interface is therefore controlled by the relative magnitude of the attractive forces of the two phases for the molecules of the *adsorbate* (substance adsorbed). Inorganic salts which are negatively adsorbed at surfaces (water-air interfaces) are usually positively adsorbed at water-liquid interfaces, and almost always so at water-solid interfaces.

This is due to a greater attraction for the solute particles by the non-water phase of the interface than the water molecules themselves exert.

Specially treated ("activated") charcoal is one of the best adsorbents known both for gases and for solutes. Many important applications are made of this property of charcoal in chemistry and technology. Gas masks for protection against poison gases in warfare owe their efficiency to the adsorptive capacity of charcoal. Colored or other soluble impurities are often removed from liquids by passing them through layers of charcoal. Solids such as charcoal which are powerful adsorbents usually possess an enormous surface area in proportion to their mass, which accounts for their effectiveness as adsorbing agents. The surface of 1 g. of charcoal has been variously estimated at from about 50 to about 600 square meters. Much of this surface is in the form of internal submicroscopic capillaries.

Electrical and Chemical Adsorption.—Adsorption in which the fundamental controlling forces are the cohesive and adhesive forces between molecules is called *mechanical adsorption* in order to distinguish it from certain more complex kinds of adsorption phenomena. Of these the most important is *electrical adsorption.*

Most surfaces in contact with water bear an electrical charge. The presence of such charges markedly influences the adsorption of ions or other charged particles. Cellulose, in common with many other substances, acquires a negative charge when immersed in water. If a strip of cellulose filter paper be arranged so that its lower end dips in a solution of eosin (a red dye) it will be observed that the eosin will rise through the filter paper almost as rapidly as the water from the solution rises by capillarity. If another strip of filter paper be similarly arranged to dip in a solution of methylene blue (a blue dye) the water will rise up the filter paper as rapidly as it does from the eosin solution, but the dye will scarcely rise at all. The difference in behavior of these two dyes is due principally to the electrical charge which their colored ions carry. The colored ions of eosin are negatively charged; those of methylene blue positively charged. The negatively charged particles of eosin are repelled from the negatively charged surface of the cellulose fibers. The eosin particles are therefore driven towards the center of the capillary columns of water in between the fibers, and move up the filter paper almost as rapidly as the water. As soon as the positively charged particles of methylene blue come in contact with the negative charges of the cellulose, however, they are held to the surface of the cellulose by the forces of electrical attraction. In other words they are electrically adsorbed. While the water rises by capillarity at about the same rate as it does from the eosin solution, the rise of methylene blue is relatively very slow.

Certain examples of adsorption have also been recognized which are designated by the name of *chemical adsorption.* Chemical reactions are involved in adsorption phenomena of this type. The familiar reaction of I_2KI with starch in which the starch stains purple is often considered to be an example of chemical adsorption.

Actually there exists no clean cut distinction between mechanical, electrical, and chemical adsorption. The several phenomena intergrade, and elements of more than one of these mechanisms are usually operating whenever adsorption occurs. Since the surface or interface of even pure water bears an electric charge, at least minor electrical effects are involved in even the simplest adsorption phenomena.

Adsorption of Water.—Water itself is strongly adsorbed at certain types of interfaces. The adsorption of water is a phenomenon which can be regarded as more nearly similar to electrical adsorption than to mechanical adsorption. Water molecules, while not charged in the sense that ions are, are strongly "polar" (Chap. X) and behave somewhat as charged bodies. Water-vapor molecules become adsorbed on many different kinds of surfaces. The water-vapor adsorbed upon glass weighing bottles often becomes an appreciable source of error in accurate quantitative work. The particles in certain colloidal systems have the property of adsorbing large quantities of water, as shown in the next two chapters. A large portion of the water which moves into various types of substances in the process of imbibition (Chap. IX) becomes adsorbed upon the internal surfaces of the imbibing substance.

The Biological Significance of Adsorption.—Adsorption phenomena are known to play a manifold role in living organisms, probably being involved in practically all cell activities. Protoplasm and many other constituents of plant cells are essentially colloidal, and adsorption phenomena are of general occurrence in colloidal systems. Within plant cells many interfaces occur, as at the boundaries between the protoplasm and vacuole, protoplasm and cell wall, and nucleus and cytoplasm, at all of which interfacial concentration of solutes undoubtedly occurs. The adsorption of certain compounds at the cytoplasmic interfaces is generally believed to exert a marked influence on the permeability of the cytoplasm. It is quite possible that interfacial tensions play an important role in the process of cell division. Imbibitional phenomena, of basic importance in the water relations of plant cells, involve the adsorption of water. The action of enzymes as well as of other catalysts is generally believed to involve adsorption phenomena. Much information regarding the structure of cells has been gained by the use of dyes which are differentially adsorbed by various constituents of cells. Chromosomes, for example, are so named because they strongly adsorb certain stains.

The relation of adsorption to many of the processes and phenomena mentioned above will receive further attention in subsequent chapters of this book. There are probably few if any processes occurring in living organisms in which adsorption phenomena are not at least indirectly involved. Adsorption and other interfacial phenomena are responsible for many of the so-called "vital" activities of living cells.

Discussion Questions

1. Describe the appearance of liquid water at and near a water-air boundary were you able to observe the individual molecules. What changes would result if the temperature were to rise 10° C.? If alcohol were added to the water? If sodium chloride were added to the water?
2. List some of the interfaces which are present in a plant cell.
3. Why aren't the nuclei of plant cells always spherical?
4. Why do two clean drops of mercury join and form a larger drop when brought into contact?
5. List some physiological processes which involve adsorption phenomena.
6. How would you expect temperature to affect the rate of adsorption? The amount of adsorbate retained at equilibrium?
7. What is the difference between adsorption and absorption?
8. Olive oil released beneath pure water will rise to the surface as large spherical drops. When released beneath a 1 per cent solution of NaOH no drops are formed, the oil rising to the surface as a thin stream. Explain.

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CHAPTER IV

COLLOIDAL SYSTEMS

Most biological problems lead ultimately to a consideration of one phase or another of the more fundamental problems of the structure, constitution, and physico-chemical properties of protoplasm. Certain characteristic physico-chemical properties of protoplasm have been recognized and studied. Protoplasm is more or less elastic. It may vary in viscosity from values not much greater than that of pure water to those of jellies. It possesses a pronounced capacity for the imbibition of water. Protoplasm is usually high in water content, but nevertheless often behaves as if immiscible with water. Mechanical disturbances may markedly influence its physical state. When exposed to very high or very low temperatures, to high concentrations of salts, or to certain other factors, protoplasm may be irreversibly coagulated. Under certain conditions it may lose its fluidity and assume a jelly-like condition, a change in physical state which is usually reversible.

Protoplasm is predominantly composed of substances in the colloidal state, and it is to these colloidal systems that it owes most of its characteristic physico-chemical properties, some of the better known of which have been listed above. The world of living organisms has, in fact, been largely molded on a colloidal pattern. Most physiological processes, reduced to their ultimate tangible mechanism, either occur in a colloidal matrix, or under such conditions as to be strongly influenced by the colloidal organization of the cells in which they take place. Many physiological processes occur only under the influence of the organic catalysts known as enzymes, which are supposed by most authorities to be in the colloidal condition and to owe many of their properties to that fact. It is impossible, therefore, to obtain any adequate comprehension of the properties of protoplasm without a background of facts and principles regarding colloidal systems. The more fundamental properties of such systems will be discussed in this and the following chapter.

Some of the most important components of the material environment of plants and animals are also essentially colloidal. Most soils contain a considerable proportion of matter in the colloidal or near-colloidal condition, and

owe many of their most distinctive properties to this fact. Few streams or bodies of water are entirely free from matter in the colloidal condition. Clouds, fogs, mists, and smoke also represent matter in the colloidal state.

General Nature of Colloidal Systems.—If a little sucrose or sodium chloride be shaken up in water the solid will soon disappear, and the resulting system will be a solution. Although there is abundant evidence which indicates that the solute particles are dispersed throughout the solvent, molecules and ions are so small that it is impossible to detect their presence by direct observation, even with the most powerful optical systems available.

If, instead of sucrose or sodium chloride, we stir some fine river bottom silt into water, a different sort of a system will result. The silt particles do not dissolve but simply become dispersed throughout the liquid. Such a system is called a *suspension*. The particles in it are of such size that they can readily be detected under a microscope. Suspensions are not stable, however, as the particles gradually settle out, and the system becomes separated into its two original components within a relatively short period of time.

Similar systems can be prepared by vigorously shaking together two immiscible liquids such as oil and water. Such systems are called *emulsions*. Emulsions are not stable unless there is also present in the system a third component called an *emulsifier*.

Still another type of system consisting of particles dispersed through water can be prepared. If a trace of sulfur be dissolved in a small volume of alcohol which is then poured into a somewhat larger volume of water, a cloudy opalescent liquid will result which is composed of sulfur *particles* dispersed through water. This type of system is intermediate in its properties between solutions and suspensions. The dispersed particles are not molecules, but, as in suspensions, aggregates of molecules. Unlike suspensions, however, such systems are relatively stable, as the particles will remain dispersed throughout the liquid indefinitely. The dispersed particles of such systems cannot be seen under a microscope, showing that they are smaller than the particles in suspensions or emulsions. They can, however, be detected with the aid of an ultramicroscope (Chap. V). The sulfur-in-water system which has just been described is a simple example of a colloidal system.

Colloidal systems, as the preceding discussion has indicated, are two-phased systems like solutions. Unlike solutions, however, the particles of the dispersed phase are not in the molecular or ionic condition, but—with certain exceptions to be noted shortly—are molecular aggregates. One colloidal particle is often composed of hundreds or even thousands of molecules lumped together. The molecular aggregates must not be so large, however, that the particles readily settle out of the system, as stability is one of the essential

attributes of colloidal systems. In general, if the dispersed particles fall within the range of 0.001—0.1 μ in diameter the system is considered a colloidal system, if larger than this a suspension or an emulsion, and if smaller a solution. The individual molecules of some substances (certain dyes, some proteins) are so large as to bring them within the colloidal range of dimensions. Hence molecular dispersions of such substances are simultaneously both solutions and colloidal systems. The limits generally accepted for the range of sizes of colloidal particles have been somewhat arbitrarily set and actually there is no sharp boundary between colloidal systems and suspensions on the one hand, nor between colloidal systems and solutions on the other. There is a perfect gradation in properties from one type of system to the next. The properties of suspensions or emulsions in which the suspended particles are of small dimensions approach those of colloidal systems, while the smaller the dispersed particles in a colloidal system the more closely it approaches a solution in its properties. The important points discussed in this section are summarized in Table 8.

TABLE 8—CLASSIFICATION OF DISPERSE SYSTEMS ACCORDING TO PARTICLE SIZE AND VISIBILITY

Type of System	Particle Size	Visibility
Suspensions and Emulsions....	Larger than 0.1 μ in diameter (Molecular aggregates)	Visible under the microscope
Colloidal Systems.....	0.001–0.1 μ in diameter (Molecular aggregates; more rarely very large molecules)	Can be detected under the ultra-microscope
Solutions.....	Smaller than 0.001 μ in diameter (Molecules and ions)	Sub-ultramicroscopic

Types of Colloidal Systems.—Colloidal systems are frequently classified on the basis of the original physical state of the two components which have been combined in forming the system (Table 9). Gas-in-gas systems do not exist, since gases do not form molecular aggregates. Of the eight types listed, the solid-in-liquid systems and liquid-in-liquid systems are of by far the greatest importance from the standpoint of living organisms.

The Nomenclature of Colloidal Systems.—The word *colloid* is derived from the Greek roots KOLLA (glue) and EIDOS (appearance). Thomas Graham, a prominent early investigator of colloidal phenomena, who did his most important work just after the middle of the nineteenth century, used this

TABLE 9—GENERAL TYPES OF COLLOIDAL SYSTEMS

Types	Examples
1. Solid-in-solid	Some alloys, certain types of colored glass, some precious stones (*e.g.* black diamond).
2. Solid-in-liquid	Many sols (see later).
3. Solid-in-gas	Smoke, fine dust clouds, certain fumes, "dust colloids."
4. Liquid-in-solid	Certain minerals and gems (*e.g.* pearls).
5. Liquid-in-liquid	Many sols (see later).
6. Liquid-in-gas	Clouds, fogs, and mists (sometimes, however, the water in such systems is condensed on minute dust particles).
7. Gas-in-solid	Some minerals. An uncommon type of colloidal system.
8. Gas-in-liquid	Some foams. An uncommon type of colloidal system.

term to designate a certain group of substances, which seemed to be set apart from other substances by several distinctive properties. When dispersed in water these substances had a slow rate of diffusion, and failed to diffuse through membranes of parchment paper. Furthermore they did not form crystals. He applied the alternate term *crystalloid* to those crystal-forming substances which, when in solution, diffused relatively rapidly and passed readily through parchment membranes. We now know that, strictly speaking, no such distinction can be made; the word colloid properly refers to a distinctive *state* of matter, and cannot be applied with accuracy to any one class of substances. Theoretically any substance can, by proper manipulation, be brought into the colloidal state, and actually this has been experimentally accomplished for a large number of substances.

Colloidal systems, as has already become evident, are composed of two phases, a continuous phase, and a discontinuous phase, the latter composed of discrete particles, each entirely separated from its fellows by the intervening continuous phase. The continuous phase is commonly called the *dispersion medium*, and the discontinuous phase the *disperse phase*. According to another terminology, applicable however only when the dispersion medium is a liquid, each individual dispersed particle is called a *micelle*, while the continuous phase of the system is called the *intermicellar liquid*.

To Graham, also, we are indebted for the terms *sol* and *gel*. A sol is a colloidal system which possesses the property of fluidity. Such systems can

be poured more or less readily from one vessel to another. To the unaided eye they often appear to be true solutions, but examination by means of an ultramicroscope reveals their colloidal nature.

Many sols "set," forming solid, but more or less elastic systems, generally called *gels*. Gelatin desserts, custards, and ordinary household jellies are familiar examples of gels. The change of a sol to gel is called *gelation*. The reverse change, as for example when gelatin is "melted" by the application of heat, is called *solation*. Some authorities use the term gel in a generic sense to include systems of the type just described, which they call *jellies*, and another type of colloidal system which they term *coagula*. The white of a hard-boiled egg is a typical coagulum.

Sols may be classified into *lyophobic* and *lyophilic* types. In the latter an appreciable affinity exists between the particles of the disperse phase and the dispersion medium; in the former no such affinity is present. When the dispersion medium is water the corresponding terms *hydrophobic* (Gr.: "water-fearing") and *hydrophilic* (Gr.: "water-loving") are employed. This last pair of terms will be used consistently in the following discussion, since from the biological standpoint colloidal systems in which water is the dispersion medium are by far the most important. The affinity between the two phases of a hydrophilic sol manifests itself by *hydration* of the micelles. Hydration is the association of one or more molecules of water with an ion, molecule, or micelle. Hydration of ions and molecules has already been discussed. Hydration of micelles is in principle the same.

Most colloidal systems composed of metallic substances dispersed in water are examples of hydrophobic sols. Gelatin, agar, starch, and gum acacia sols are familiar examples of hydrophilic systems. Protein sols also belong in this group. Actually all possible gradations exist from highly hydrophilic sols to highly hydrophobic sols.

Suspensions.—The general nature of suspensions has already been indicated. Suspensions are not generally regarded as playing a very significant role in living organisms. The particles of suspension size which frequently can be observed in the protoplasm are apparently composed entirely of relatively inert materials. Particles of suspension size are very common in soils, and as such are an important part of the environment of the roots of plants. A consideration of suspensions is also of importance in developing the conception of the structural and dynamic aspects of colloidal systems proper.

Emulsions.—Emulsions are systems in which one liquid is dispersed throughout another with which it is virtually immiscible, the particles of the dispersed liquid exceeding about 0.1 μ in diameter. While other types of emulsions are theoretically possible all such systems encountered in common

experience fall naturally into two groups generally known as *oil-in-water* emulsions, and *water-in-oil* emulsions. In the former type an oil or some other liquid insoluble in water, or practically so, is dispersed throughout a water dispersion medium. In the latter type the converse is true, the oil or other liquid immiscible with water constitutes the dispersion medium, while small aggregations of water molecules are dispersed through it. The proportions of the components of most emulsions can be varied between wide limits. Emulsions are not generally considered to be true colloidal systems, but, like suspensions, approach them in properties. Emulsions occur commonly in the cells of plants and animals, and are generally believed to be essential components of the protoplasmic matrix. Both water-in-oil and oil-in-water emulsions are known to exist in living cells, but the latter type is commoner. When examined under high magnification with a microscope protoplasm in its grosser aspects often presents the appearance of an emulsion of fats and fat-like substances dispersed through the body of the protoplasm.

Some common examples of oil-in-water emulsions are milk, cream, emulsions of olive oil in water, and the latex of the rubber tree, milkweed, etc. Perhaps the only generally familiar water-in-oil emulsion is butter. Many other similar systems can be prepared, however. Water in olive oil is one example of such a system. Water in kerosene is another. The word "oil" as employed in this discussion is not restricted to those substances usually classified chemically as oils, but is here somewhat generalized to include other liquids which are immiscible with water. Kerosene, for example, is not chemically an oil.

Emulsions, with the exception of some very dilute ones, lack stability unless there is also present in the system an *emulsifier*. In the absence of an emulsifier, the two components of an emulsion rapidly separate, the oil, being the component of lower specific gravity, rising to the top. The group of substances classified as emulsifiers is chemically a very heterogeneous one. Some of the best known emulsifiers are the soaps, saponins, various substances which form hydrophilic colloids when dispersed in water (gums, gelatin, etc.), and fine suspensions of certain rather inert materials such as sulfur, carbon, silica, and resin. The last group is probably of little importance in living organisms.

Oil-in-water emulsions may be stabilized by such substances as soaps of the alkali metals (Na, K, Li, etc.), gum acacia, and proteins; while soaps of the alkaline earth metals (Ca, Ba, etc.), and gum dammar are examples of stabilizers of water-in-oil emulsions. In general, emulsifying agents which are soluble in water or form hydrophilic sols in water, stabilize oil-in-water emulsions while those which are insoluble in water but soluble in oil stabilize

water-in-oil emulsions. Many pharmaceutical preparations are stabilized by means of gum acacia or gum dammar.

Emulsions found in nature are usually stabilized by proteins. This is true of milk and cream, in which the droplets of fat are dispersed throughout a water medium. The latex of plants, one of the best examples of a naturally occurring emulsion, is also stabilized by proteins. Mayonnaise dressing is essentially an emulsion of olive oil in water, stabilized by the proteins of the egg which is the third essential component of the system. Protoplasmic emulsions are probably also stabilized by proteins, although soaps may also act as emulsifiers in plant cells.

The mechanism of the stabilizing effect of emulsifiers upon emulsions is not well understood, and it is probable that it varies with different types of emulsifiers. It is well known, however, that any substance which greatly lowers the interfacial tension between water and another liquid will usually stabilize an emulsion composed of those two liquids. The usual interfacial tension between water and benzene, to cite one example, is about 35 dynes per centimeter. Addition of a little sodium oleate (a soap) to water will lower this interfacial tension to about 2 dynes per centimeter. Sodium oleate is an excellent stabilizing agent for an emulsion of benzene in water.

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CHAPTER V

THE PROPERTIES OF SOLS AND GELS

The discussion in this chapter will deal only with sols in which water is the dispersion medium. Such systems are classified into the two groups of hydrophilic sols and hydrophobic sols. The dispersed particles in all such colloidal systems fall within the size range of 0.001 to 0.1 μ, but their size may vary greatly with different sols. Even in a given sol the particles may exhibit a wide range of dimensions, although in some they are quite homogeneous in size.

Most of the important differences between hydrophilic and hydrophobic sols are due to the hydration of the dispersed particles in systems of the former type. There is no general agreement regarding the exact physico-chemical relationship between the micelle and its water of hydration; but only two conceptions have any wide currency at the present time. One of these relates this hydration to an actual solution of some of the water in the substance of the micelles (Fig. 4, *A*).

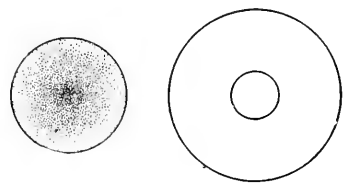

FIG. 4. Diagrammatic representation of possible relationships between a micelle and its water of hydration: (*A*) solution of water in the micelle. The proportion of dissolved water present is represented as decreasing toward the center of the micelle. (*B*) orientation of the water molecules as a "shell" around the micelle.

A more probable theory, however, holds that no actual solution of the medium in the micelles occurs, but that water molecules are oriented around each dispersed particle forming a "shell" many layers of molecules in thickness (Fig. 4, *B*). It is presumed that the first layer of oriented water molecules is so firmly attached to the micelle that it is essentially an integral part of it. The successively enveloping layers of water molecules are also oriented, but with increasing distance from the periphery of the micelle the forces of attraction and orientation progressively decrease. In this zone there is a gradual transition from water molecules which are practically all oriented to those which are completely unoriented. Oriented molecules fit together more closely than unoriented molecules. They are "packed" more

closely, just as more bricks can be stacked in a given space if arranged regularly, than if tossed in indiscriminately. As a result of this packing the water in these oriented shells has a greater density than that in the bulk of the liquid; in other words there is an actual contraction in the volume of the liquid associated with the micelles.

The fact that micelles may, under certain conditions, lose their water of hydration very rapidly would seem to favor the latter theory. It is possible, of course, that in some hydrophilic systems the water is actually dissolved in the micelles, that in others it is present only as a shell of oriented molecules, while in still others both of these two suggested modes of hydration may exist.

The important properties of sols will now be summarized, with special attention to differences between the properties of sols of the hydrophilic type and sols of the hydrophobic type.

Dilution.—Most sols are extremely dilute. In other words the actual mass of substance dispersed throughout the dispersion medium is extremely small in proportion to the total volume of the system. The well-known colloidal gold sols, for example, rarely contain more than 1 g. of gold dispersed per liter of sol. Other hydrophobic sols are correspondingly dilute. Most hydrophilic sols are also extremely dilute, although there are some exceptions to this statement, as very viscous sols of such substances as starch and gum acacia can be prepared which contain 10 parts or more of dispersed material per 100 parts of sol.

Slow Rate of Diffusion of the Dispersed Particles.—In general the particles which make up the disperse phase of a sol diffuse much more slowly than substances in true solution, *i.e.* in the molecular or ionic state. The slow rate of diffusion of micelles as compared with most ions and molecules is clearly correlated with the relatively large size of the colloidal particles. There is, however, no sharp line of demarcation in terms of diffusion rates between colloidal micelles and solutes. The diffusion rates of some solutes with large molecules are not perceptibly faster than those of the dispersed particles of colloidal systems in which the micelles are relatively small in size.

Filterability.—Sols are usually filterable; that is they pass through ordinary filter papers without any appreciable separation of the disperse phase from the dispersion medium by the filter. Usually there is some loss of the disperse phase due to an initial adsorption when the sol first comes in contact with the filter. Sometimes this may be very considerable. Since the pores in ordinary filter papers are about 2-5 μ in diameter, and even porcelain filters, such as those widely used in bacteriological work, have pores 0.2-0.6 μ in diameter, it is easy to understand why micelles with diameters in the size range 0.001-0.1 μ pass through.

Special filters have been devised, however, with pores of such a small diameter that the disperse phase can be separated from the dispersion medium by filtering a sol through them. Such filters are known as *ultrafilters*. The process of filtering through such a filter is known as *ultrafiltration*. The most commonly used types of ultrafilters are those made of collodion or gelatin. The size of the pores in such filters can be controlled by the length of time allowed for drying and in other ways. It is possible to prepare ultrafilters with pores of such dimensions that solutes can pass through them, but colloidal micelles cannot.

Tyndall Phenomenon.—Suppose that a clean glass vessel, preferably one with flat, parallel sides, be filled with pure water and held so that a strong pencil of light passes laterally through the vessel. If an observation be made laterally and at right angles to the path of the beam of light, no distinctive trace of its path through the water can be detected. Such a liquid is said to be optically empty. The same will be true if the water in the vessel be replaced by a sugar or salt solution, or in fact, by any true solution.[1] Suppose, however, that the vessel be filled with a hydrophobic sol and observed in the manner just described. The results are strikingly different. The path of the light will be clearly delineated as a cloudy, often opalescent track through the sol. Even a colloidal system which seems perfectly transparent to the unaided eye will usually show some turbidity when submitted to this test. The intensity of this effect varies greatly with the specific colloidal system, and with the concentration of the disperse phase, but it is universally shown by hydrophobic sols.

The phenomenon just described is called the *Tyndall phenomenon*, and is due to the scattering or diffraction of light. The difference in the index of refraction between the two phases of the colloidal system is also a factor determining the intensity of the Tyndall effect. The greater this difference, the stronger the effect. Since in the diffraction of light, the short wave lengths (blue end of the spectrum) are bent more than the longer wave lengths, a partial separation of the spectrum results. For this reason a sol with a colorless disperse phase often appears to be pale blue when viewed in the path of a strong beam of light.

Similar Tyndall phenomena are exhibited by hydrophilic sols, but usually the effect is less striking when sols of this type are viewed in the path of a beam of light than the effect observed when hydrophobic sols are employed.

[1] Actually a trace of the light track will usually be perceived even in water or true solutions, due to the presence of contaminating dust particles. In order to prepare a truly optically empty liquid, provision must be made for the removal of such dust particles.

The instrument known as the ultramicroscope is based on the principle of the Tyndall phenomenon. The limit of the resolving power of ordinary microscope is about 0.1 μ. The ultramicroscope can be used for detecting the presence of particles in the size range 0.001 μ to 0.1 μ, *i.e.* in the colloidal range. It is not possible to observe colloidal particles directly in the ultramicroscope; only the light diffracted from their surfaces can be seen. Neither can any definite image of particles in this small range of sizes be obtained. The ultramicroscope is a microscope which is so arranged that the colloidal system or other material to be examined can be illuminated laterally (*i.e.* at right angles to the tube of the microscope). This lateral illumination is usually provided by a powerful source of light and a suitable series of condensing and focussing lenses, so arranged that the light is focussed to a point within the mount. Under the ultramicroscope the dispersed particles of a hydrophobic sol appear as bright spots of light varying in size and brilliancy. Very little concerning the actual size or shape of the micelles can be determined since each bright spot represents merely the light diffracted by a single particle. It is possible, however, to determine the *number* of particles in a given volume of solution by means of the ultramicroscope. The use of the ultramicroscope consists essentially in an observation of the Tyndall phenomenon in a small volume of a sol under the microscope.

Brownian Movement.—In 1828, the botanist Robert Brown observed through a microscope that pollen grains which were suspended in water showed a rapid oscillatory motion. Brown at first was inclined to attribute this motion to the fact that the pollen grains were alive, but examination of preparations of dead pollen grains and spores showed that they likewise exhibited such a motion. It became evident therefore that this movement was in no way connected with living processes. We now know that any particle up to about 4 or 5 μ in diameter will exhibit this movement when suspended in a liquid. This phenomenon is termed *Brownian movement*, after its discoverer.

Many suspensions in which the particles are within the range of microscopic visibility exhibit Brownian movement. It is clearly shown by many of the smaller species of bacteria when suspended in water. In solid-in-gas colloids, such as tobacco smoke, the dispersed particles show a very vigorous Brownian movement. Particles in the protoplasm of slime molds and certain other species frequently exhibit a Brownian movement which is clearly discernible under the microscope. For particles of a given mass, the smaller their volume the greater the amplitude of their Brownian movement. For particles of equal volume, the less their mass the more vigorously they will exhibit Brownian movement. In general this phenomenon is exhibited more

clearly by the micelles of hydrophobic sols than by those of hydrophilic sols. The viscosity of the liquid phase is also an important factor governing the rapidity with which the dispersed particles move. The more viscous the liquid, the more sluggish the movement of the particles.

Brownian movement is caused by the kinetic activity of the molecules of the solvent. Even the smallest particles in which Brownian movement can be observed are very large in proportion to the size of the solvent molecules which impinge upon them. A particle suspended in a liquid such as water suffers a continual bombardment by the molecules of the liquid. If the particle be relatively large, at any given moment it is bombarded on every side by numerous molecules, moving in all possible directions, and at different speeds. The effects of the individual impacts largely counteract each other, however, and there is little or no movement of the particle. If the particle be smaller, however, the results are quite different. At any given moment a much smaller number of water molecules impinge upon the particle. The resulting forces cease to be balanced and the sum total effect of the blows which the particle sustains on some one side are greater than the effect of the blows sustained on any other side. Hence the particle moves. The next moment a greater impetus may be given to the particle from some other direction and the course of its movement is changed. In this way the highly erratic movements of suspended particles known as Brownian movement originate. Increase in temperature increases the rate of Brownian movement because of an increase in the kinetic energy of the solvent molecules. This phenomenon is the nearest approach we have to actual visible evidence of the validity of the kinetic theory of matter. It almost brings before our eyes the veritable "dance of the molecules."

Osmotic Pressure.—As discussion in Chap. VIII shows the osmotic pressure of solutions is a colligative property; that is one which depends on the proportion of solute particles (molecules or ions or both) to solvent molecules, regardless of the kind of solute particles. A sol should also possess an osmotic pressure since theoretically a micelle has the same effect upon the magnitude of the osmotic pressure as a molecule or an ion. A brief discussion should aid in making this point clear. Large molecules have the same theoretical effect upon osmotic pressure as small molecules. The individual molecules of some compounds are large enough to fall within the colloidal range of sizes. They are both molecules and micelles at one and the same time. Such a micelle should have just as much of an effect on osmotic pressure as a molecule. Actually the osmotic pressures of sols never exceed a small fraction of an atmosphere and are therefore much less than the osmotic pressure of any but the most extremely dilute solutions (Chap. VIII). The osmotic pressure

of colloidal systems depends upon the concentration of the particles of the disperse phase. In most sols the total mass of the disperse phase is not only relatively small; but it is present in the form of particles which are much larger than molecules or ions. The negligible osmotic pressures of sols are a necessary corollary of the relatively small concentration of dispersed particles.

Viscosity.—The viscosity of a fluid is its resistance to flow. The more viscous a liquid the less readily it will flow. Glycerine, for example, is much more viscous than water. The viscosity of hydrophobic sols never varies appreciably from that of the dispersion medium—water. Unlike hydrophobic sols the viscosity of hydrophilic sols is usually greater than that of the dispersion medium. The viscosity of hydrophilic sols increases appreciably with increase in the concentration of the sol, but the relation is not a linear one (Fig. 5). The rapid increase in the viscosity of hydrophilic sols with increasing concentration is ascribed to the hydration of the micelles. Increasing the concentration of the disperse phase results in a decrease in the relative amount of free water present due to the association of a larger proportion of the water with the micelles. This reduces the "fluidity" of the sol, hence raises its viscosity.

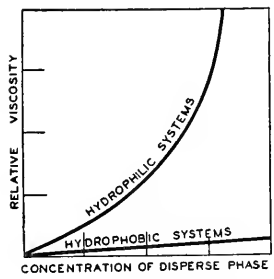

FIG. 5. Relation of relative viscosity of hydrophobic and hydrophilic sols to the concentration of the disperse phase.

The viscosity of all liquids, including sols, is influenced by temperature. In general, increase in temperature decreases viscosity. In hydrophilic systems this reduction in viscosity with increase in temperature is probably due to two factors: the decrease in viscosity of the medium itself, and the decrease in the hydration of the micelles.

Electrical Properties.—The dispersed particles of all *hydrophobic sols* carry electrical charges. A colloidal system, however, is electrically neutral, because for every charge carried on a micelle an equal charge of opposite value is carried by ions in the dispersion medium. The situation is similar to that in a solution of an electrolyte. Although the individual ions are charged, for every negative charge carried by an anion an equal positive charge is carried by a cation. In some colloidal systems the dispersed particles are negatively charged, in others positively charged, but normally all the dispersed particles in any one system bear a charge of the same sign.

Whatever the origin of the micellar charges they invariably are produced in such a way as to involve the release of ions into the dispersion medium which may therefore be regarded as also being charged. When the micelles

are negatively charged, the dispersion medium is positively charged and *vice versa*. Electrostatic attraction therefore exists between the surface charges of a colloidal particle and the ions of opposite charge in the dispersion medium. The result is that surrounding each colloidal particle with its charged surface is a "shell" of ions of opposite charge.

This arrangement of charges at the surface of a micelle is called an *electric double layer*. Similar electric double layers exist also at boundaries between solid surfaces and liquids, as for example along the walls of a capillary tube. Figure 6 represents the distribution of the electrical charges around two micelles, one positively charged, one negatively charged. The innermost layer of ions in the dispersion medium is probably compactly oriented around the oppositely charged micelle, which is in turn surrounded by progressively more diffuse layers. That is, while most of the ions are close to the charged surface of the micelle, some are farther away, although with increasing distance from

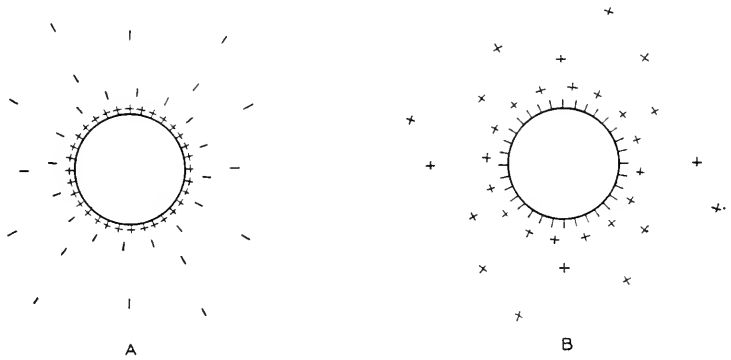

FIG. 6. Diagrammatic representation of the electric charges around micelles: (*A*) positively charged, (*B*) negatively charged.

the surface of a micelle, the number of ions associated with that micelle decreases rapidly.

The ions of the double layer are in dynamic equilibrium with undissociated molecules at the periphery of the micelle proper. Anions and cations of the double layer are continually uniting and forming uncharged molecules which become part of the micelle. Contrariwise molecules at the surface of the micelle are continually dissociating into cations and anions. If the micelle is negatively charged the anions adhere to its surface; if positively charged, the cations. The ions of the opposite charge become part of the outer shell around the micelle.

The presence of this electrical double layer results in a difference of elec-

trical potential between the micelle and the intermicellar liquid. The magnitude of this difference of potential varies with different sols and with the same sol under different conditions.

The usual charges borne by the micelles of some of the better known hydrophobic sols are shown in Table 10.

TABLE 10—SIGN OF THE ELECTRICAL CHARGE ON THE PARTICLES OF CERTAIN HYDROPHOBIC SOLS

Positively Charged	Negatively Charged
1. Metallic hydroxide sols ($Fe(OH)_3$; $Al(OH)_3$)	1. Metal sols (Sn, Pt, Ag, etc.)
2. Methyl violet	2. Sulfide sols (As_2S_3, PbS)
3. Magdala red	3. Mastic sols
4. Bismarck brown	4. Sulfur, selenium, and tellurium sols
	5. Indigo

The usual method of determining the sign of the charge upon the dispersed particles of sols is to observe the direction of their migration in an electrical field. If two electrodes are so arranged as to dip into a hydrophobic sol contained in a suitable vessel, and the electrodes connected with a direct current of proper potential, it will be found that the dispersed particles will move toward one of the poles. By suitable arrangements, using a microscope or ultramicroscope (depending upon the size of the particles), the migration of the particles may be actually watched. In a positively charged ferric hydroxide sol, for example, the micelles will move towards the negatively charged electrode, while in a negatively charged arsenious sulfide sol they will move towards the positively charged pole. This phenomenon is known as *cataphoresis* or *electrophoresis*.

As a micelle moves under the influence of an electric current only the innermost layer of the electric double layer—the one which determines its electrical charge—clings to the micelle and moves with it. This inner layer slides past the oppositely charged ions of the outer shell of one micelle after another as it moves towards the anode if negatively charged, or towards the cathode if positively charged. Simultaneously ions of the outer layer move towards the opposite pole from the one towards which the micelles migrate. There is a close analogy between this phenomenon and the behavior of the ions of an electrolyte during electrolysis.

The sign of the charge on the dispersed particles of any sol can be determined by cataphoresis. Particles considerably above the colloidal range of dimensions frequently can be shown to exhibit cataphoretic migration. The phenomenon can be demonstrated with bacteria, unicellular algae, spores, etc. Practically all such small living organisms are negatively charged.

The dispersed particles of hydrophobic sols apparently acquire their charges either by electrolytic dissociation or by adsorption. In some such systems the charges seem to arise as a result of ionization of some of the molecules composing the micelle. The ions released into the dispersion medium become the outer envelope of the double layer, leaving the micelle with a residual and compensating charge of ions of opposite sign. Individual molecules of some substances are large enough to fall within the colloidal range of sizes. The dye Congo Red, which is the sodium salt of a complex organic acid, is an example of such a substance. Dispersed in water this compound produces sodium ions and a colloidal anion, the latter, of course, being negatively charged.

In other systems the electrical charge on the dispersed particles is apparently acquired by adsorption of either the positive or negative ions of an electrolyte, the ion of opposite charge becoming the outer shell of the double layer. Ferric hydroxide sols [2] are normally positively charged. The charge on the micelles of these sols is often ascribed to the adsorption of Fe^{+++} ions of the $FeCl_3$ from which ferric hydroxide sols are usually prepared, the Cl^- ions forming the outer layer around the micelles. Similarly the negative charge of the micelles of arsenious sulfide sols is often ascribed to the adsorption of H_2S which is used in the preparation of this sol. Dissociation of H_2S results in the release of H^+ ions into the dispersion medium leaving the dispersed particles with a residual and compensating negative charge.

Similarly it is believed that some substances acquire an electrical charge by adsorbing hydrogen or hydroxyl ions—more frequently the latter—from their water dispersion medium. Certain inert substances such as cellulose, carbon, quartz, collodion, etc., are believed to become charged in this way. All of these substances acquire a negative charge when in contact with water, indicating that the hydroxyl ions are adsorbed, the hydrogen ions becoming the outer shell of the double layer.

The micelles of some *hydrophilic sols* are charged; those of others are not. As in hydrophobic systems the dispersed particles of different hydrophilic sols acquire their charges in different ways. In some the charges on the particles

[2] Most investigators believe that the so-called ferric hydroxide sol is actually a sol of hydrated ferric oxide ($Fe_2O_3(H_2O)_x$).

originate by ionization of some of the surface molecules of the micelles. The electrical charges on the micelles of protein sols arise in this way. In other systems the charges may originate from traces of electrolytes which are present as impurities. This is probably true of agar sols. Such charges may be regarded as similar in their origin to those acquired by adsorption on the micelles of hydrophobic sols, in that the charge is due to some compound associated with the substance of which the micelle is composed, and not to that substance itself. Most of the better known hydrophilic sols are negatively charged.

Flocculation.—Since the dispersed particles of any sol are in rapid motion it would seem that repeated collisions would result in a progressive agglomeration of the particles into larger and larger masses which eventually would settle out of the system. Sols, however, are relatively stable systems, and it is important to understand the mechanism by which the stability of such colloidal systems is maintained.

The stability of *hydrophobic sols* is maintained almost entirely by the charge which each micelle carries. Although by Brownian movement the dispersed particles are repeatedly brought close together actual collision seldom occurs because the shells of ions around the micelles are mutually repellent.

Reduction of the charge on the micelles of any hydrophobic sol to the point where there is no difference of electrical potential across the double layer results in agglomeration of the individual particles into flakes of a size which rapidly settle out of the surrounding liquid. This phenomenon is called *flocculation, coagulation,* or *precipitation.* The point at which there is no difference of electrical potential across the double layer is known as the *isoelectric point* of the sol. At the isoelectric point the micelles of a sol are, relative to the surrounding medium, completely uncharged. As, by Brownian movement, two such micelles are brought into contiguity they no longer repel each other with sufficient intensity to prevent their agglomeration. By the addition of other micelles such particles rapidly increase in size, soon resulting in flocculation of the sol. Merely reducing the electric charge to a value approaching that of the isoelectric point is sufficient to induce instability and slow flocculation in many sols.

Flocculation is most commonly initiated by the introduction of electrolytes into the system. Very small quantities of an electrolyte are often sufficient to cause the flocculation of a relatively large volume of sol. The important principles regarding the flocculation of hydrophobic sols by electrolytes can be most easily elucidated by a discussion of some of the data in Table 11.

The flocculating effect of an electrolyte is due primarily to the added ion

of opposite charge from that borne by the colloidal particle. Thus the As_2S_3 sol may be flocculated by cations such as Na^+, Ca^{++}, or Al^{+++}, while the $Fe(OH)_3$ sol may be flocculated by anions such as Cl^-, NO_3^-, or SO_4^{--}.

TABLE II—FLOCCULATING EFFECT OF ELECTROLYTES ON HYDROPHOBIC SOLS (DATA OF FREUNDLICH, 1903.) THE CONCENTRATION OF ELECTROLYTES IS THE MINIMUM WHICH RESULTS IN COMPLETE FLOCCULATION

Negatively charged arsenious sulfide sol. (15.57 millimols As_2S_3 per L.)		Positively charged ferric hydroxide sol. (10.3 millimols $Fe(OH)_3$ per L.)	
Electrolyte	Concentration (millimols per L.)	Electrolyte	Concentration (millimols per L.)
LiCl	81.5	NaCl	9.25
NaCl	71.2	KCl	9.03
KCl	69.1	KNO_3	11.9
KNO_3	69.8	K_2SO_4	0.204
$MgCl_2$	1.00	$MgSO_4$	0.217
$MgSO_4$	1.13	$K_2Cr_2O_7$	0.194
$BaCl_2$	0.964		
$Ba(NO_3)_2$	0.959		
$CaCl_2$	0.905		
$AlCl_3$	0.130		
$Al(NO_3)_3$	0.137		

Furthermore, the flocculating effect increases with an increase in the valency of the effective ion. The first part of Table II shows that the trivalent cation Al^{+++} is more effective in flocculating the negatively charged As_2S_3 sol than the bivalent cations Ca^{++}, Ba^{++} or Mg^{++}, which in turn are much more effective than the univalent cations K^+, Na^+, and Li^+. Similarly the second part of the table shows that the flocculating effect of bivalent anions (SO_4^{--}, $Cr_2O_7^{--}$) upon a positively charged $Fe(OH)_3$ sol is greater than that of univalent anions (Cl^-, NO_3^-).

However, the influence of the valency of an ion upon its flocculating effectiveness does not follow a simple 1 : 2 : 3 arithmetical ratio. It took, as shown in Table II, about 88 times as much NaCl as $CaCl_2$, and about 7 times as much $CaCl_2$ as $AlCl_3$ to accomplish complete flocculation of the As_2S_3 sol.

In the flocculation of hydrophobic sols the ion of the added electrolyte with a charge of the same sign as that of the micelle is not entirely without effect. The influence of such ions is usually to increase the stability of the system. Precisely stated, therefore, the influence of an electrolyte upon the

micelles of a hydrophobic sol is a differential effect between the anions and cations but the influence of the ion of opposite charge predominates.

The mechanism of the flocculation of a hydrophobic sol is too complex a process to be considered in more than a general way in an introductory discussion. In general flocculation results from destruction of the double layers around the micelles. There is a close analogy between the flocculation of a hydrophobic sol by an electrolyte and a precipitation reaction between one electrolyte and another. The micelles of hydrophobic sols may be regarded as giant ions bearing numerous charges.

When $CaCl_2$ is added to a solution of Na_2SO_4 slightly dissociated $CaSO_4$ is precipitated, leaving paired Na^+ and Cl^- ions in the solution. An analogous phenomenon results when $CaCl_2$ is added to a negatively charged As_2S_3 sol in which the outer shells of the double layers are composed of hydrogen ions derived from the dissociation of adsorbed H_2S. The resulting flocculant consists of uncharged particles of As_2S_3 + Ca, while the H^+ and Cl^- ions left behind in the solution pair off in the usual manner, forming hydrochloric acid. In other words Ca^{++} ions have replaced the H^+ ions in the outer shells of the micelles. The result, however, is an unstable particle, since Ca^{++} ions do not remain in the dissociated state when associated with As_2S_3 micelles which owe their negative charge to the dissociation of adsorbed H_2S molecules. They combine with such micelles, thus dissipating their electrical charges. Flocculation of the uncharged micelles then ensues.

Flocculation may also be initiated by introducing into a hydrophobic sol another hydrophobic sol with micelles bearing a charge of opposite sign. If $Fe(OH)_3$ sol be slowly added to an As_2S_3 sol a point will be reached at which complete flocculation will occur. The particles of the two sols will settle out as an intimate mixture. The same phenomenon occurs when any negatively charged hydrophobic sol is added to any positively charged hydrophobic sol in sufficient quantity, or *vice versa*. This process is called *mutual flocculation*. When this phenomenon occurs the ions of the outer zone of one kind of particle pair off with the oppositely charged ions of the outer shell of the other kind.

The addition of a small amount of a hydrophilic sol, such as a gelatin or gum arabic sol, to a hydrophobic sol makes flocculation of the latter by electrolytes or micelles of opposite charge difficult or impossible. This effect of a hydrophilic on a hydrophobic sol is termed *protective action*. Protective action is apparently due to the adsorption of the micelles of the hydrophilic sol around the micelles of the hydrophobic sol. The properties of the sol, therefore, become essentially those of the hydrophilic system. As will be seen

shortly hydrophilic sols are much less easily flocculated by electrolytes than hydrophobic sols and this is undoubtedly the basis for protective action.

We turn now to the question of the mechanism of the flocculation of *hydrophilic sols*. The micelles of such sols may or may not carry an electrical charge, but whether charged or not such colloidal systems are stable. Hydrophobic sols, as already shown, are stable only when the micelles bear an electrical charge. One of the most important differences between hydrophobic and hydrophilic sols is the possession by the latter of a second stability factor, which in itself is effective in keeping such sols stable for long periods of time. In our previous discussion we have seen that uncharged micelles of hydrophobic sols soon agglomerate and settle out of the dispersion medium. Why do not the uncharged micelles of a hydrophilic sol behave in the same way? This is probably due to the effect of hydration upon the properties of the dispersed particles. The micelles of all hydrophilic sols, it will be remembered, are highly hydrated. The first layer of molecules of water of hydration is probably so firmly bound to the particle as to virtually constitute an integral part of the micelle itself. Surrounding this are other layers of water molecules more or less completely oriented depending on their distance from the surface of the particles. Each such particle is "cushioned" against impacts with other particles by its enveloping shell of oriented water molecules. Agglomeration of the micelles is thus prevented, and hence even uncharged hydrophilic sols are stable.

The possession of two stability factors by hydrophilic sols complicates the mechanism of flocculation in such systems. The manner in which flocculation of hydrophilic sols may occur can be illustrated by reviewing a simple experiment. The experimental material is a dilute (about 0.1 per cent) sol of agar-agar. Such a sol is perhaps the most typical example of a simple hydrophilic system. If a relatively large volume of alcohol be added to a small portion of such a sol, the micelles lose their water of hydration, and the sol acquires the cloudy, bluish, opalescent appearance typical of many lyophobic sols. In fact it now *is* a lyophobic sol, and shows all the typical properties of such systems. The alcohol, which is a powerful dehydrating agent, has robbed the micelles of their shells of oriented water molecules. Nevertheless, the sol is still stable, since the micelles retain their original negative charges. Finally, let a drop of a solution of an electrolyte such as $AlCl_3$ be added to the sol. The sol now flocculates almost immediately since the only remaining stability factor—the electrical charge—has been destroyed by the addition of the electrolyte, and the opalescent cast of the sol disappears.

The stability factors of a hydrophilic sol can also be dissipated in the opposite order. Suppose that the $AlCl_3$ solution first be added to the agar

sol until the charges on the micelles are neutralized. Although now at its isoelectric point, unlike hydrophobic systems, the sol does not flocculate. If, however, alcohol now be added to the system, immediate flocculation occurs, because of a dehydration of the micelles, resulting in an elimination of the only remaining stability factor in the system.

Briefly then, in order to flocculate a hydrophilic sol, its micelles must be both dehydrated and electrically discharged, except in the occasional systems in which micelles are uncharged, in which dehydration alone will suffice. Dehydration alone of a hydrophilic sol with charged micelles results in its conversion into a lyophobic sol.

The inter-relationships among the factors involved in the stability and flocculation of hydrophilic sols are shown in Fig. 7 which is self-explanatory.

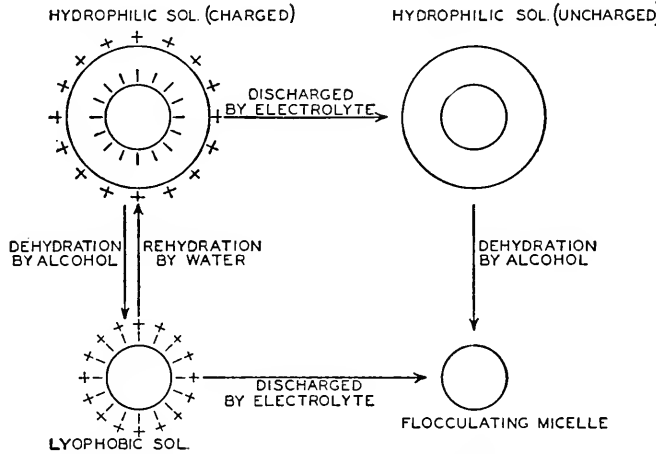

FIG. 7. Diagrammatic representation of the flocculation of a micelle of a hydrophilic sol.

The addition of relatively large quantities of certain electrolytes to hydrophilic sols will result in their flocculation without any previous removal of the water of hydration of the micelles by means of alcohol or any other dehydrating agent. Only very soluble salts are effective in this way. It is evident that this phenomenon, usually called *"salting out,"* involves a more complicated reaction than that taking place in simple flocculation and must be distinguished from the latter phenomenon. Three salts which are especially suitable for salting out hydrophilic sols are $(NH_4)_2SO_4$, $MgSO_4$, and Na_2SO_4. In these three salts the ions, especially the anions, acquire relatively large quantities of water of hydration. The result of the addition of a very

strong solution of such a salt to a hydrophilic sol is a two-fold one. A small initial amount of the added electrolyte discharges the micelles. Addition of further increments of an electrolyte eventually brings about dehydration of the micelles due to the great attraction of the added ions for water, or to the effect of the solute in reducing the diffusion pressure of the water (Chap. IX), or to both, and the resultant separation of the dispersed phase out of the system. Salting out, therefore, consists in an electrical discharge of the micelles, followed by their dehydration. It results in the destruction of both of the stability factors of the system.

Amphoteric Properties of Protein Sols.—Protein sols differ from most others in that the micelles are amphoteric, *i.e.* they may act either as an acid or as a base. The acid properties of proteins depend upon their $-COOH$ groups; their basic properties upon their $-NH_2$ groups (Chap. XXVI). Whether the proteins will combine with acids or bases depends principally upon the pH of the dispersion medium. In a gelatin sol, for example, in which the pH of the medium is above the isoelectric point[3] the $-COOH$ groups of the molecules react with a base such as sodium hydroxide, forming "sodium gelatinate." This compound then dissociates into sodium ions and negatively charged gelatin micelles. If the pH of the medium is below the isoelectric point the $-NH_2$ groups of the gelatin molecules may combine with the molecules of an acid such as HCl forming "gelatin hydrochloride." Dissociation of this compound produces chloride ions and positively charged gelatin micelles.

Like the micelles of other sols, those of proteins are uncharged with respect to the medium at the isoelectric point. Therefore no migration of the micelles occurs if an electric current is passed through a protein sol at its isoelectric point. At pH values higher than the isoelectric point protein micelles migrate towards the anode, while at values below the isoelectric point they migrate towards the cathode.

The principles governing the stability and flocculation of a protein sol are similar to those which hold for other hydrophilic sols with the one further complication that protein micelles may be either positively or negatively charged. Protein sols are stable at their isoelectric point because, although uncharged, they possess, like all hydrophilic sols, micelles which are highly hydrated. On either side of the isoelectric point the micelles of a protein sol have the additional stability factor of an electrical charge. Addition of a sufficient quantity of a dehydrating agent such as alcohol to a gelatin sol at its isoelectric point will result, as it does with an uncharged agar sol, in immediate flocculation. If the micelles are charged, however, addition of alcohol will

[3] The pH value of the isoelectric point of gelatin is about 4.7.

result not in flocculation, but in the conversion of the system into a lyophobic sol. Addition of a suitable electrolyte to this sol will result in a discharge of the particles and their consequent flocculation. These relations are shown diagrammatically for a protein sol in Fig. 8.

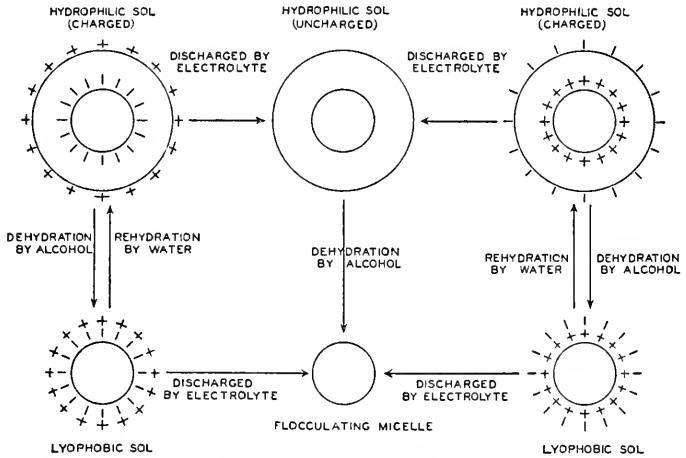

FIG. 8. Diagrammatic representation of the flocculation of a micelle of a protein sol.

Properties of Gels.—Under certain conditions most hydrophilic sols change into gels. Any gelatin sol which is not too dilute, for example, will "set" upon standing and form a gel. Everyone is familiar with such gelatin gels, often as they appear on the table under the guise of desserts. Other familiar gels are the agar gels widely used as a medium for the culturing of bacteria, fungi, algae, etc., ordinary household "jellies" which are basically pectin gels, and starch gels. The latter also sometimes come before our eyes by the dessert route, under the name of corn starch puddings. Some hydrophilic sols, however, do not ordinarily form gels. This is true of sols of gum acacia and of some protein sols.

The gel-forming capacity of some substances is very remarkable. A gelatin sol containing as low a proportion as one part of gelatin to one hundred of water will usually gelate. Agar gels containing only 0.15 per cent of agar can be prepared. In such a gel one part of agar has the property of removing the liquidity from nearly 700 parts of water.

Since gels form from two-phase colloidal systems, it is generally assumed that they also are two-phase systems. In all of the gels which we shall have occasion to consider, the liquid phase is water, although gels in which one component is some other liquid are well known. Gels are usually rigid

enough to maintain their shape under the stress of their own weight. This means that they will be molded to the shape of the vessel in which the gelation has occurred, and will retain the shape of that vessel after being removed from it.

Solutes diffuse through gels almost as rapidly as through pure water, unless the gels are very concentrated. The diffusion of a solute through a gel is easily demonstrated by a simple experiment. A gelatin sol is allowed to solidify in a test tube which is then inverted in a shallow dish containing a solution of a dye such as methylene blue. Within 24 hours diffusion of the dye into the gelatin gel can be detected. Because of the fact that there are no convection currents in a gel to complicate the results, this is perhaps the best visual method of demonstrating the diffusion of solutes.

Two equal quantities of an electrolyte, one dispersed in water, the other in a gel of equal volume, will conduct an electric current almost equally well. In other words ionic mobility is apparently as great when the ions are dispersed in a gel as when they are dispersed in pure water.

The velocities of chemical reactions occurring in a gel medium are not appreciably different from the velocities of the same reactions occurring under the same conditions of solute concentration, temperature, etc., in a water medium. Neither of the last two general statements are strictly true for very concentrated gels.

The above three properties of gels distinguish them clearly from the solid or amorphous states of matter. These properties must be reconciled with any acceptable theories of the structure of gels.

Elastic Gels.—Two general types of gels are usually recognized, the elastic type, and the non-elastic type. The best known example of the latter is the silica gel. Elastic gels are the important type biologically. Gelatin-water and agar-water gels are probably the best-known examples of this type of colloidal system. When such a gel dries a gradual and consistent shrinkage in its volume ensues until desiccation is complete. After desiccation the dry matter of the gel will imbibe water, but no other liquid. Gels in which the liquid phase was other than water will imbibe, after desiccation, only the liquid originally present.

Elastic gels are generally heat reversible. When heated such gels are converted into sols (*solation*) and when cooled such sols resume their gel condition (*gelation*). Usually this reversal of state can occur a number of times to a colloidal system without greatly affecting its physical properties when in either the sol or gel condition. The temperatures at which the solation and gelation of a given colloidal system occur are not identical. The processes differ in this respect from the melting and freezing of a solid. For

example, a 4 per cent gelatin sol gelates at about 28° C, but the resulting gel must be raised to about 31° C. before solation will occur. For agar gels the temperature spread between gelation and solation is much greater. The former process occurs at approximately 40° C.; the latter at about 85° C. The viscosity of a heat reversible sol increases steadily with a decrease in temperature and shows no sudden change as the sol passes into the gel condition.

The Structure of Gels.—Numerous theories purporting to explain the structure of gels have been advanced, and there is no doubt that there are elements of truth in most of them. Since it seems improbable that the molecular architecture of all gels is the same, it is unlikely that any one of the proposed theories will apply equally well to all gels. The structure of gels is too fine to be resolved by the microscope, and only rarely has the ultramicroscope revealed anything when it has been used as an instrument for studying gel structure. Necessarily, therefore, most evidence of the structure of gels is indirect.

There seems to be little doubt that in non-elastic gels of the silicic acid type that the solid phase is crystalline, and forms a sort of a rigid framework. The liquid phase is held in the interstices of this solid framework. In some respects this may be regarded as the simplest type of gel structure.

One of the older theories of the structure of elastic gels is the so-called "honeycomb theory." According to this theory the fluid phase of the gel is discontinuous, being separated into minute chambers or compartments, bounded on all sides by films of the solid, or at least of a more solid phase. The analogy between such a cellular structure and a honeycomb is obvious. The "solid" phase is not necessarily supposed to be composed of the chemically pure, originally dispersed material; it may represent simply a phase which is relatively rich in the dispersed substance as compared with the fluid phase. In a gelatin gel, for instance, it might be supposed that the solid phase is relatively rich in gelatin but poor in water while the converse is true for the fluid phase.

At the present time an hypothesis more generally favored is that both the solid and liquid phases of a gel are continuous. The solid phase is usually visualized as a meshwork of long, tangled fibrillae of ultramicroscopic, or sub-ultramicroscopic dimensions; the spaces within this interwoven mesh being occupied by the fluid phase. This theory is often called the "brushpile" theory in allusion to the supposed jumble of intermeshing threads of the solid phase. The remarks made in the preceding paragraph concerning the constitution of the two phases are also valid for this theory.

This last theory appears to be most acceptable in the light of the known

facts regarding the very slight effect of gels upon diffusion, conductivity, and the velocity of chemical reactions. Ultramicroscopic examination of certain gels has also yielded evidence which appears to support this theory.

Since elastic gels can be readily transformed into hydrophilic sols, and hydrophilic sols into gels, there is good reason for believing that hydrophilic sols may also possess a fibrillar structure. Such a structure is also postulated by many authorities for protoplasm, as later discussion will show.

Hysteresis.—The statement is sometimes encountered that gels possess the faculty of "memory." This statement is not, of course, to be accepted literally. It is merely a way of saying that the previous treatments to which a gel has been subjected have an influence, often marked, upon its behavior, so that in an allegorical sense the gel may be said to "remember" those treatments. This phenomenon of the influence of the previous treatment of a gel upon its behavior is known as *hysteresis*.[4] It is well illustrated by the following experiment of Gortner and Hoffman (1927). Three gelatin gels were prepared containing respectively 10, 20, and 40 g. of gelatin per 100 cc. of water. Strips of these gels of equal rectilinear dimensions and thickness were then dried in a current of warm air until all of them were reduced to a moisture content of about 3.5 per cent. In other words the three gels were all brought into what would superficially seem to be identical physical conditions. The dried sections were then placed in distilled water and allowed to imbibe water, weighings being made from time to time. The results are shown in Fig. 9.

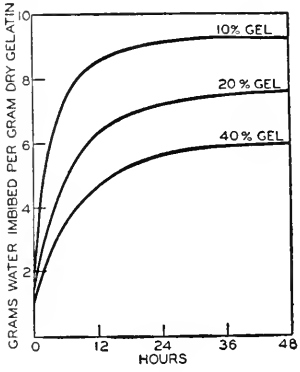

FIG. 9. Curves illustrating hysteresis in gelatin gels. Data of Gortner and Hoffman (1927).

In spite of the fact that all three of these gels possessed the same water content when immersed in water their swelling behavior was quite different, depending in each sample on the previous history of the gel. The gel which was originally prepared in the proportion of 10 parts of gelatin to 100 of water swelled the most, followed in order by the one originally prepared in a proportion of 20 parts of gelatin to 100 of water, and finally by the gel originally prepared in a proportion of 40 parts of gelatin to 100 parts of water.

The example cited is just one of the many hysteresis effects which have been recognized in gels. All sorts of factors—mechanical, thermal, electrical,

[4] This use of the term hysteresis should not be confused with its common use in another sense in physics and engineering.

and even the factor of time may induce such effects in gels. It follows that in experimental work with gels, if results are to be comparable, all the gels used in a given experiment or set of experiments must have had identical previous histories.

Syneresis.—*Syneresis* may be defined as the spontaneous separation of a portion of the liquid component of a gel. The liquid which separates is not quite pure, however, being essentially a very dilute sol. Syneresis is a widely observed phenomenon. It may be observed in both gelatin and agar gels. The so-called "bleeding" of agar culture media is familiar to all who work with them. The liquid which usually forms around gelatin gels which have stood for some time is often a result of syneresis. It has been suggested that several important biological phenomena involve the process of syneresis. These include the formation of vacuoles in plant and animal cells, the separation of serum from a blood clot, the exudation of serum into a blister, glandular secretion, and the contraction of muscles.

Thixotropy.—If a trace of sodium chloride is added to a test tube full of 10 per cent bentonite (a colloidal clay), and vigorously shaken, a colloidal sol will result which will set to a gel after standing a few minutes. By shaking, this gel can be converted to a sol which will again set upon brief standing. The process may be repeated an indefinite number of times. This phenomenon is called *thixotropy*. Protoplasm also exhibits thixotropic reactions. Stirring of the protoplasm or subjection of a cell to pressure can be shown to greatly reduce its viscosity and probably induces gel to sol changes. Thixotropic phenomena may therefore play important roles in cellular physiology.

Discussion Questions

1. What are the three most outstanding characteristics of colloidal systems in which water is the dispersion medium?
2. The charges on colloidal micelles are assumed to be balanced by electrostatically equal charges in the dispersion medium. If this is true why do micelles exhibit cataphoresis?
3. How can you determine whether a given organic dye forms a solution or a sol when dispersed in water?
4. Given a clear colloidal sol how would you determine whether it was hydrophilic or hydrophobic? Whether its micelles were positively or negatively charged?
5. List some colloidal systems found in plant cells.
6. Why are not the micelles of hydrophobic sols flocculated by the ions of opposite sign in the double layer?
7. Why are not the protoplasmic colloids of root hair cells flocculated by the cations and anions that enter them from the soil?

Suggested for Collateral Reading

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CHAPTER VI

PLANT CELLS

The Structure of Plant Cells.—The typical cell of the higher plants is a tiny compartment enclosed by a tough elastic wall (Fig. 10). The wall of cells consists of two major parts: (1) The *middle lamella* and (2) the *primary wall* (Kerr and Bailey, 1934). In walls of many plant cells a third structural component, the *secondary wall,* is also present. Although some plant cells are known which do not have a well defined cell wall, this structure is so generally present in plant cells as to be considered one of their characteristic features.

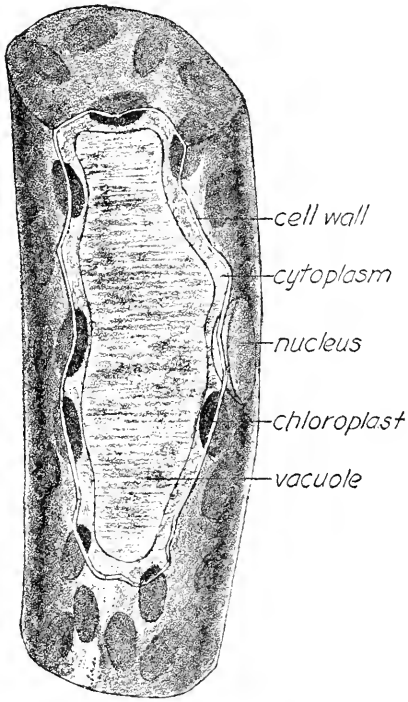

FIG. 10. Perspective view of a palisade cell from the leaf mesophyll.

Lining the interior of the wall and occupying more or less of the cell cavity is the *protoplasm.* The protoplasm of active cells is a transparent, slightly viscous, granular material that lacks any conspicuous structural background. It is not homogeneous, however, and contains a number of definite structures. One of these, the *nucleus,* is a denser body which is more or less spheroidal in shape and is separated from the remaining protoplasm by a definite membrane, the *nuclear membrane.* Within the membrane surrounding the nucleus are: (1) a clear liquid known as the *nuclear sap,* (2) a delicate network of denser material, the *reticulum,* and (3) one or more small spherical masses of material known as the *nucleolus* or *nucleoli.*

All of the protoplasm outside of the nucleus of the cell constitutes the

cytoplasm. In a typical mature plant cell the cytoplasm is present as a thin layer lining the inner surface of the cell wall. The two boundary layers of the cytoplasm—that in contact with the cell wall and that in contact with vacuole—are called the *cytoplasmic membranes* (Chap. X). Imbedded in the cytoplasm are numerous well differentiated bodies known as *plastids.* Plastids are specialized cytoplasmic structures which are usually centers of certain types of physiological activity. They are commonly classified on the basis of their color into three groups: The *leucoplasts* which are colorless, the *chloroplasts* which contain the green chlorophyll pigments (also yellow pigments), and the *chromoplasts* which contain red or yellow pigments. *Chondriosomes*, minute rod-like or granular bodies, are also found in the cytoplasm. The significance of these structures is not positively known, although a number of different roles have been ascribed to them.

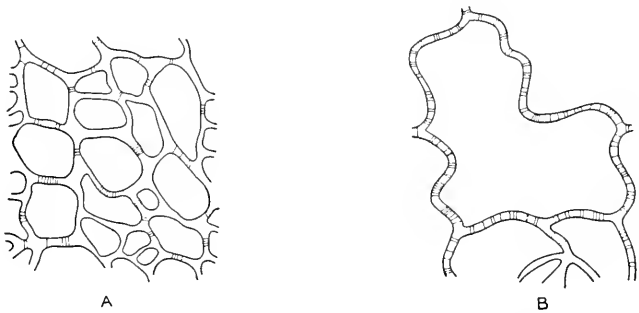

FIG. 11. Plasmodesms in cell walls of tobacco: (*A*) sieve tubes and companion cells, (*B*) epidermal cells of leaf. Redrawn from Livingston (1935).

Although the cell wall appears to imprison each protoplast, and to effectively isolate it from the protoplasm of adjoining cells, actually there is probably a continuation of protoplasm from cell to cell. By certain techniques it can be demonstrated that minute pores extend from cell to cell through the cell walls. These pores often contain cytoplasmic strands which connect the cytoplasms of adjacent cells. These strands are termed *plasmodesms* (Fig. 11). Livingston (1935) has demonstrated the occurrence of plasmodesms in the walls of cells from a number of different tissues of the tobacco plant, and it is generally supposed that they are of widespread if not universal occurrence in plant cell walls.

The bulk of the interior of mature plant cells is occupied by a single large cavity, the *vacuole*, which is filled with *cell sap.* The cell sap is composed of water in which a great variety of substances are dissolved or colloidally dispersed.

There is no general agreement among cytologists regarding the exact classification of the parts of plant cells. The vacuole, for example, is frequently classified as a part of the protoplasm because it first appears as minute droplets in the protoplasm of very young cells. Physiologically, however, the vacuole of the mature cell is as distinct an entity as the protoplasm or cell wall and it is therefore considered as a separate part of the cell in this book. The following classification includes the principal parts of a mature plant cell. A few of these parts, as for example the various kinds of plastids, do not occur in every cell.

- The Mature Plant Cell
  - Cell Wall
    - Middle lamella
    - Primary Wall
    - Secondary Wall
  - Protoplasm
    - Cytoplasm
      - Plasmodesms
      - Cytoplasmic membranes
      - Undifferentiated cytoplasm
      - Plastids
        - leucoplasts
        - chloroplasts
        - chromoplasts
        - elaioplasts
      - Chondriosomes
    - Nucleus
      - Nuclear membrane
      - Nuclear sap
      - Reticulum
      - Nucleolus
  - Vacuole
    - Cell Sap
      - Water
      - Various compounds in solution or in a state of colloidal dispersion
    - Crystals

The Origin and Development of Cells.—As soon as it became clear that all plant and animal tissues were composed of cells the question of the origin of cells naturally arose. This proved to be a difficult problem for the pioneer

investigators and for many years there was much disagreement over the question. The now universally accepted principle that cells can arise only by the division of pre-existing cells was first demonstrated beyond any reasonable doubt by Nägeli just before the middle of the Nineteenth Century. A number of methods are now known by which this division is accomplished but a detailed discussion of them is beyond the scope of this book. In higher plants cell division occurs chiefly in certain restricted regions called *meristems*. The production of new cells involves not only the division of pre-existing cells, but the subsequent enlargement and maturation of their cell progeny. The morphological and physiological aspects of these stages in the development of cells are discussed in Chap. XXXI.

The Forms and Sizes of Cells.—All newly formed cells do not differentiate morphologically in the same way. Some elongate parallel to the axis of growth more than in other directions and thus produce the longer fiber cells that make up much of the xylem and phloem tissues. These cells commonly develop walls that are greatly thickened. Some cells enlarge about equally in all directions forming *isodiametric* cells, the walls of which are never greatly thickened. Cells of this type are present in the pith, leaf mesophyll and in other parenchymatous tissues. According to Lewis (1935) both plant and animal cells are basically tetrakaidecahedrons; *i.e.*, 14 sided, although many other geometrical shapes are found, especially in specialized types of cells. An almost endless variation in cell shapes and sizes may be seen in the tissues of any vascular plant, all of which may develop from similarly shaped meristematic cells. Many of these are figured and described in greater detail in later chapters.

In size cells show an equally great range. Most plant cells have diameters that fall somewhere between 10 μ and 100 μ. A single cubic centimeter of tissue may, therefore, contain millions of cells. Some cells, however, are much larger. Certain varieties of cotton, for example, produce fibers that commonly attain a length of 4 cm. while the phloem fiber cells of *Boehmeria nivea* are known to exceed 55 cm. in length. Such cells have lengths that are many thousand times their diameters.

The Cell Wall.—One of the most important features of plant cells is the presence of a conspicuous cell wall that encloses the living protoplasm.

There are great variations in the thickness of the walls of different kinds of cells and even greater differences in the physical and chemical properties of cell walls. In fact the walls of plant cells exhibit such great contrasts in structure and chemical composition that it is difficult to single out any specific cell wall as having a structure that may be considered typical of plant cell walls in general. There are, however, certain features of the cell walls of the

vascular plants that are almost invariably present. Probably the most important of these is the almost universal presence of cellulose as the structural framework of the wall. A second feature of plant cell walls is that the cellulose seems invariably to possess a crystal-like structure. No plant cell wall, however, is composed solely of cellulose. There is present in every wall greater or lesser quantities of one or more other substances in addition to cellulose. The most important of these are the pectic compounds, lignin, hemicelluloses, cutin, and suberin. A third characteristic of most cell walls is their lamellate structure. Most cell walls seem to be an aggregation of numerous delicate lamellae of varying physical and chemical properties, all of which are firmly welded together forming the superficially homogeneous wall.

Origin and Development of the Cell Wall.—In the vascular plants the division of a plant cell is preceded by the division of the nucleus. Nuclear division is a complicated process involving organization of the nuclear reticulum into unit chromosomes, splitting of those chromosomes, migration of one of the chromosomes of each pair to opposite ends of the cell, and finally, reconstitution of a daughter nucleus from each set of daughter chromosomes. Just after the final stage ("telophase") of mitosis a membrane develops in the center of the cell and extends its margins until it makes contact on all sides with the existing cell wall, thus forming a septum which separates the protoplasts of the newly formed cells (Fig. 12). This first membrane, the *middle lamella*, does not contain cellulose in quantities that permit its detection but seems largely, if not entirely, composed of colloidal pectic compounds. During the enlargement of the cell a thin layer composed largely of cellulose is deposited from the cytoplasm of each young cell on the two sides of the middle lamella. This *primary wall* contains large amounts of pectic materials in addition to cellulose and may vary in thickness and in physical properties at different times of the year. Primary walls may become considerably thickened as is the case in the collenchyma cells of young stems. As long as a cell wall possesses a marked elasticity and exhibits reversible changes in thickness or in other physical properties it is classed as a primary wall. The walls of cambium cells, parenchyma cells and collenchyma cells are all examples of primary cell walls.

After the enlargement of the cell ceases the deposition of cellulose on the inner surface of the primary wall may continue until the wall becomes conspicuously thickened. When, as in most cases, this thickening is accompanied by an almost complete loss of the elasticity of the wall the added material is known as the *secondary wall*. Secondary walls are further characterized by the absence of reversible changes in thickness and by their low content of pectic compounds. They are largely composed of cellulose which is frequently

associated with lignin, hemicelluloses, or other membrane substances. In extreme cases secondary thickening may continue until the wall occupies most of the interior of the cell. The formation of a secondary wall prevents any further enlargement of the cell. All cells have a middle lamella and a primary wall, but secondary walls are present only in certain types of cells.

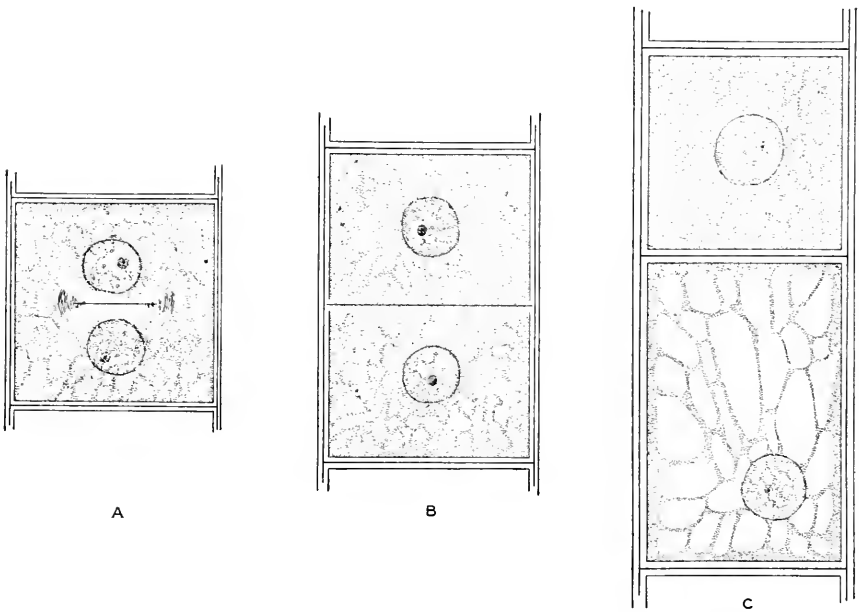

FIG. 12. Diagram illustrating formation of a plant cell wall. (*A*) cell plate between two daughter nuclei, (*B*) cell plate (middle lamella) completely separating the daughter cells, (*C*) primary wall has been formed on each side of the middle lamella. One of the newly formed cells has undergone considerable enlargement.

Phloem fibers, stone cells, tracheids, and wood fibers are typical examples of cells with prominent secondary walls.

Increase in thickness of the wall usually appears to take place by the addition of definite layers of cellulose or other cell wall constituents to the inner surface of the existing wall. In most walls these layers are too thin to be detected without swelling or otherwise treating the wall.

The Structure of the Cell Wall.—The basic unit in the structural organization of the cellulosic framework of the plant cell wall is the cellulose "molecule." Cellulose "molecules" are not units of definite molecular weight but long chains of varying length formed by the condensation of at least a

100-120 and possibly many times this number of β d-glucose molecules[1] (Chap. XXII).

Studies of cellulose walls with the X-ray and polarized light have furnished evidence that the "molecules" of cellulose are aggregated into bundles known as *micelles*. The micelles have been estimated upon the basis of X-ray photographs to have a diameter of approximately 5 or 6 $m\mu$ and a length of about 60 $m\mu$. A unit of this size would consist of about sixty parallel cellulose chains each being made up of about 120 glucose units (Fig. 13). It is probable that in many cell walls long chain-like "molecules" formed by the condensation of other sugars are associated with the cellulose chains in the micelles (Norman, 1938).

Originally the micelles were believed to be well defined units which were cemented together by some non-crystalline material. Recent work indicates,

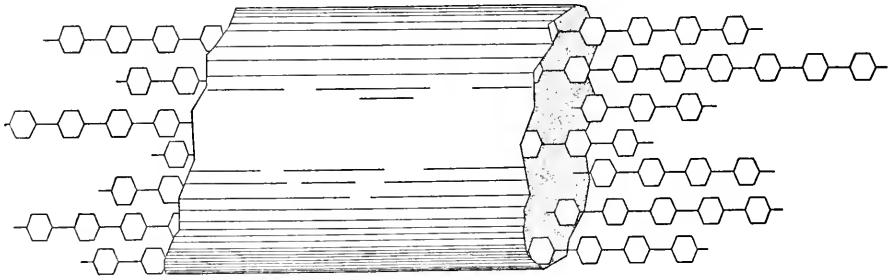

FIG. 13. Diagram illustrating structure of a small portion of a cellulose micelle. Parts of only a few of the constituent cellulose molecules are shown.

however, that many of the cellulose chains are much longer than the micellar aggregates and extend from one micelle to another, thus welding the micelles into a coherent anastomosing system. It is also probable that the micellar aggregates vary in size. Cellulose walls are no longer regarded, therefore, as a system of discrete crystalline units bound together by some cementing substance but rather as a structure composed of molecular aggregates (micelles) which are welded together by interlocking cellulose molecules.

There seems to be little doubt regarding the presence of intermicellar spaces. The spaces, indicated in black in Fig. 14, form an interconnecting system between the anastomosing micelles. In most primary cell walls the

[1] Using the ultracentrifuge method Stamm (1930) reports that cellulose molecules contain some 250 glucose residues while Kraemer and Lansing (1935), using the viscosity method, estimate that native plant cellulose contains approximately 1300 glucose residues.

intermicellar spaces are filled with pectic compounds; in woody tissues they are filled with lignin, and in cutinized walls with waxes and cutin. In walls that are almost pure cellulose, such as the secondary wall of cotton fibers, it is possible that the intermicellar spaces are filled chiefly with water.

The smallest *visible* units of cellulose walls are delicate threadlike strands or *fibrils*. In primary walls these fibrils form a loose anastomosing network, the meshes of which are usually filled with colloidal pectic compounds. In secondary walls the fibrils are often grouped into coarser strands which wind around the cell in a steep spiral the angle of which may vary in different layers and even in different parts of the same layer (Fig. 15).

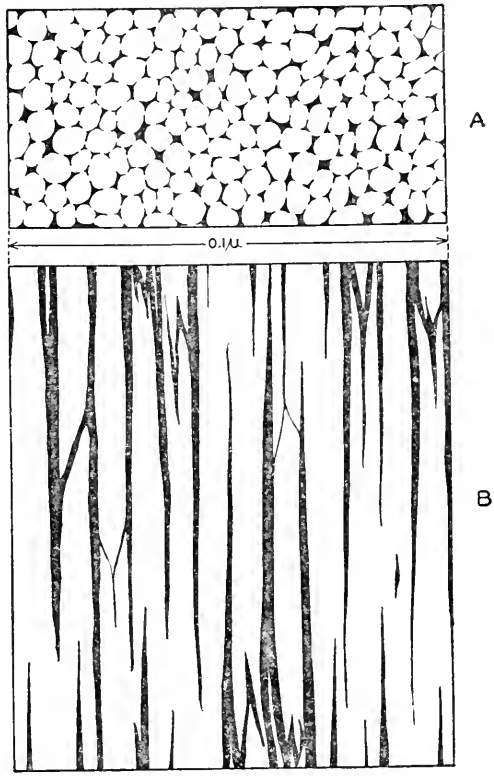

FIG. 14. Micellar structure of walls. Black areas represent intermicellar spaces; white areas represent the cellulose micelles. (*A*) cross section, (*B*) longitudinal section. Redrawn from Frey-Wyssling (1936).

The Physical Properies of Cell Walls. — The physical properties of primary cell walls differ from those of secondary walls. These differences may be ascribed to the greater abundance of cellulose in secondary walls and to differences in the structural organization of the cellulose in the two kinds of walls. Both primary and secondary walls are transparent to wave lengths of the visible spectrum and both are usually quite permeable to most substances dissolved in water.

Secondary cell walls composed predominantly of cellulose possess tensile strengths that compare favorably with those of steel. The breaking strength of a flax fiber, for example, may be as great as 110 kg. per mm.^{2} of wall area while the tensile strength of a hardened spring steel ranges between 150 and 170 kg. per mm^2. Although the tensile strength of primary walls is considerably less than that of secondary

walls it is only rarely that they are subjected to strains in excess of their breaking strength.

Primary cell walls are usually quite elastic. The mesophyll cells of the leaves of some species, for example, are known to undergo reversible changes in volume of 30 per cent or more in response to changes in turgor pressure. Secondary walls, on the other hand, are elastic only within very narrow limits and break suddenly when their tensile strength is exceeded. The elasticity of primary walls is undoubtedly related to the loose open meshwork of cellulose strands that compose it, while the close parallel orientation of the cellulose micelles in secondary walls prevents any appreciable elongation without rupture.

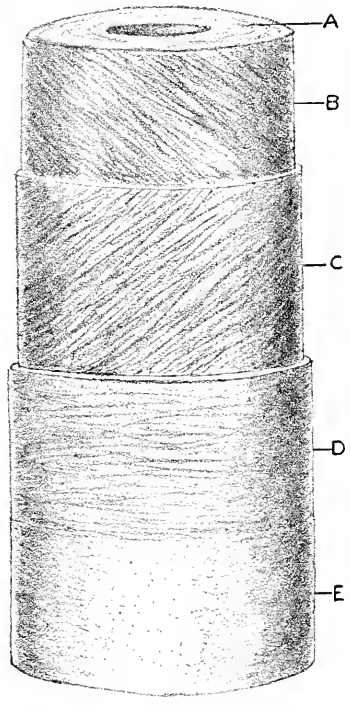

FIG. 15. Diagram illustrating the structure of a thickened plant cell wall. (*A*) inner layers of secondary wall, (*B*) second layer of the secondary wall, (*C*) first layer of the secondary wall, (*D*) cellulose framework of the primary wall, (*E*) primary wall of cellulose and pectic compounds. Note that the fibrils may be differently oriented in different layers of the same wall.

Chemical Constituents of Cell Walls. —Cellulose is by far the most abundant compound found in the cell walls of the higher plants. Associated with cellulose in all the primary cell walls of vascular plants are greater or lesser amounts of *pectic compounds*. Their presence in the middle lamella and in the intermicellar spaces of the primary walls has already been noted. The chemistry of the pectic compounds is discussed in Chap. XXII.

Lignin is an important constituent of the walls of most of the cells that make up woody tissues and it also occurs commonly in other thickened walls. Although lignin can be isolated from cell walls by suitable treatments as a brownish amorphous substance it is probably altered considerably in the process. Its structural formula and molecular weight are unknown. Since the lignins produced in different species appear to differ in their chemical properties it seems very probable that lignin is not a compound of definite molecular weight but a complex mixture of a number of chemically similar substances. The lignin of spruce wood has been extensively studied

and several workers have suggested that its molecular weight lies between 800 and 900. Brauns and Hibbert (1933) have proposed the empirical formula $C_{47}H_{52}O_{16}$ to represent the structural unit of spruce lignin but no empirical formula has received general acceptance.

Lignin first appears in the middle lamella and primary wall and later can be detected in the secondary wall. When associated with cellulose, lignin is present in the spaces between the micelles and not within the micelles. Lignified walls are usually freely permeable to water and solutes. The tensile strength of lignified cell walls is the same as that of cellulose walls but lignified walls resist compression better than cellulose walls. The increased resistance of lignified walls to compression is explained by the assumption that the presence of lignin in the intermicellar spaces welds the cellulose micelles into a single coherent mass and thus prevents the bending and buckling of the cellulose strands when they are subjected to compression strains.

Cutin is the name applied to the mixture of wax-like materials found on the outer surface of the epidermal cell walls of leaves, stems, fruits, and other organs. These wax-like substances are intimately associated with cellulose and often with pectic substances producing a wall of great structural complexity (Meyer, 1938). Cutinized cell walls are relatively impermeable to water. The presence of cutin in the outer walls of epidermal cells greatly reduces the evaporation of water from the surfaces of plant tissues.

Suberin is similar in many of its properties to cutin. It constitutes an important part of cork cell walls and it is also found in the walls of a few other specialized types of cells. Most of the surface of perennial plants, aside from the leaves and very young stems is covered with suberized cell walls. Such walls are relatively impermeable to water. The chemistry of both cutin and suberin is discussed in Chap. XXIII.

Hemicelluloses are a poorly defined group of polysaccharides associated with cellulose in plant cell walls. They are not chemically related to cellulose, as the name implies, but possess very different chemical and physical properties. The chemistry and metabolic significance of these substances are discussed in Chap. XXII.

Callose is the name given to a carbohydrate membrane substance found in the perforated septa ("sieve plates") of the sieve tubes. Similar material, presumably of the same chemical composition, has been found in pollen grains and constitutes the inner layer of pollen tubes. It has also been reported as occurring in the fungi. The exact chemical composition of callose is unknown since it has never been obtained in sufficient amounts to permit quantitative determinations.

Chitin, a nitrogenous substance common in the exoskeleton of insects, is a constituent of the walls of many fungi and bacteria. It has been reported as being present in the walls of certain algae but it is unknown in any of the higher plants.

Tannins (Chap. XXII) are commonly found in the cell sap but they also occur in the walls of certain tissues, especially cork and wood cells.

Mucilages (Chap. XXII) are common constituents of the outer walls of many water plants and occur also in the outer walls of some seed coats, in glandular hairs and in other specialized tissues.

Inorganic compounds such as silica and salts of calcium, iron, and other metals are also present in some plant cell walls. None of these inorganic compounds, however, are regarded as essential constituents of the cell wall.

Protoplasm.—In most mature cells of the vascular plants protoplasm is present only as a thin layer covering the inner surface of the cell walls but in some specialized cells branching strands of protoplasm also extend across the vacuole. Under high magnification the protoplasm of active cells appears as a colorless fluid, in which are suspended numerous tiny granules and droplets of insoluble materials. These granules frequently exhibit active Brownian movement. The fluid component of protoplasm also is frequently in motion, streaming around the inner surfaces of the cell walls. Embedded in this fluid component, and carried passively by it, are the specialized bodies known as plastids.

Although protoplasm appears to be a simple liquid, no simple liquid could possibly possess the remarkable powers of synthesis, assimilation, reproduction, growth and sensitivity that characterize the protoplasm of living plant cells. The properties and behavior of protoplasm clearly show that it is not a substance but that it must be regarded as a complex system of substances. This system is dynamic; it is constantly undergoing changes yet at the same time the changes are so regulated and controlled that the system is not disrupted. A cell is alive only so long as the organization of this dynamic protoplasmic system is maintained.

The protoplasmic system of living plant cells almost invariably contains a well differentiated globular body known as the *nucleus*. All of the protoplasm outside of the nucleus is designated as *cytoplasm*. Although the nucleus is separated structurally from the cytoplasm by a delicate membrane the two components are not physiologically isolated. The nucleus and the cytoplasm appear to be mutually dependent and there is good reason to believe that the nucleus controls and regulates the physiological processes that occur in the cytoplasm. Probably because of the close relationship between the nucleus and the cytoplasm many biologists use the terms "protoplasm" and "cyto-

plasm" synonymously. In this book, however, the terms will be used as has been indicated earlier in this chapter.

The Chemical Composition of Protoplasm.—Since protoplasm is a dynamic system of substances it is not possible to subject it to chemical analysis without destroying it. In the strict sense, therefore, it is impossible to discover the chemical composition of protoplasm. It is possible, however, to examine the substances present after the protoplasmic system has been destroyed and to determine their chemical composition and relative abundance. A number of such studies have been made.

Water is the chief component of all physiologically active plant protoplasm usually making up more than 90 per cent of the system. The water content of the protoplasm of dry seeds, on the other hand, may be less than 10 per cent.

Most attempts to determine the chemical composition of plant protoplasm have been made upon species of the myxomycetes. At certain stages in their life history these organisms consist of naked masses of labile protoplasm. They are often found "flowing" over rotten logs in damp woods. The fact that the myxomycetes provide relatively large quantities of protoplasm entirely free from cell wall material has made them a favorite object for chemical analysis. Even in such organisms, however, not all of the constituents of the plant body can be regarded as integral parts of the protoplasm. Distributed throughout the protoplasmic mass are particles of foods and other inert materials which cannot be separated from the protoplasm. The results of a chemical analysis of the dry residue of a myxomycete plasmodium are shown in Table 12.

As shown in this analysis proteins and other nitrogen-containing compounds constitute the bulk of the organic matter in the plasmodium of this species. Many different varieties of proteins are known to occur in the protoplasm of plant cells. They are compounds of enormous molecular weight (Chap. XXVI), and undoubtedly make up a large proportion of the labile structural framework of the protoplasm.

Lipids (Chap. XXIII) constitute a smaller fraction of the protoplasm than the proteins. Three types of lipids occur in the protoplasm; the true fats (oils), the phosphatides (phospholipids) and the sterols, of which phytosterol is an example. The oils are generally suspended in the protoplasm in the form of minute globules. They are probably more important as food reserves than as actual constituents of the protoplasm. The phospholipids and sterols, on the other hand, are believed to be essential constituents of the protoplasmic system.

The water-soluble carbohydrates, amino acids, etc., present in the plas-

modium of this species are probably almost entirely foods. The inorganic compounds ("mineral matter") in plant cells are chiefly the phosphates, chlorides, sulfates, and carbonates of magnesium, potassium, sodium, and calcium.

TABLE 12—ANALYSIS OF THE PLASMODIUM OF A MYXOMYCETE RESEMBLING FULIGO VARIANS. (DATA OF LEPESCHKIN, 1923)

	Percentage of dry weight
A. Water soluble substances, chiefly from vacuoles:	
Monosaccharides	14.2
Proteins	2.2
Amino acids, asparagine, etc.	24.3
B. Insoluble organic substances, principally constituents of the protoplasm:	
Nucleo-proteins	32.3
Nucleic acids	2.5
Globulin	0.5
Lipo-proteins	4.8
Neutral fats	6.8
Phytosterol	3.2
Phosphatides	1.3
Other organic matter	3.5
C. Mineral matter, about half water soluble	4.4

An analysis such as that presented in Table 12 is not without value inasmuch as it furnishes some information regarding the kinds and proportions of the compounds present in protoplasm. It supplies no more information regarding the *organization* of protoplasm, however, than a chemical analysis of the ground up debris of a wrecked house would furnish regarding the structure of that house. This point is worthy of emphasis, since with the progress of biology it becomes clearer and clearer that the properties of protoplasm are as much a function of its physiochemical organization as of the specific kinds of compounds present. Although we commonly refer to "protoplasm" as the essential constituent of all living cells, it is evident that there are at least as many different varieties of protoplasm as there are species of plants and animals. All of them, however, are dynamic systems composed of the same types of compounds, and all of them possess a colloidal organization of a complex type.

The Physical Properties of Cytoplasm.—Although it is not possible to explain adequately the mechanisms responsible for the physiological activities of cytoplasm it is possible to study the behavior of cytoplasm under various

conditions. Studies of this kind have furnished considerable information about some of its physical properties.

1. *Transparency.*—Like the walls of most living cells protoplasm is usually transparent to the wave lengths of the visible spectrum. Photochemical reactions can therefore occur in cells located well below the surfaces of many plant organs such as leaves.

2. *Elasticity.*—Some authorities consider cytoplasm to be highly elastic. Seifriz (1936) has shown that it is possible to draw out the cytoplasm of living plant cells into long threads which behave as elastic bands and snap back into the cytoplasmic mass when released (Fig. 16). It is difficult to determine, however, whether the elastic properties of such cytoplasmic threads are characteristic of the cytoplasm as a whole or only of the membranes which enclose it. The phenomenon may be due to surface tension forces rather than to a true elasticity of the cytoplasm proper. Experiments designed to

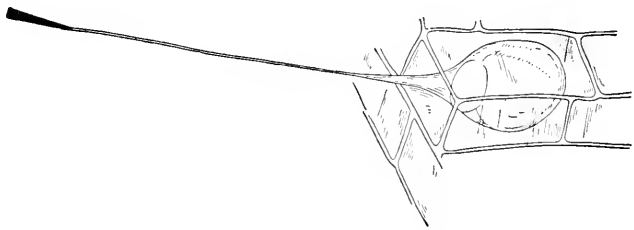

FIG. 16. Demonstration of the "elastic" property of cytoplasm. Redrawn from Seifriz (1936).

measure the elasticity of the cytoplasm within its limiting membranes indicate very low values. Probably active cytoplasm as a whole possesses elastic properties because of the presence of elastic surface membranes but the bulk of the cytoplasm which lies within these membranes behaves as a liquid and is essentially non-elastic. The entire protoplasm of a cell may become elastic, however, when the viscosity is greatly increased, particularly when it is in the gel state.

3. *Viscosity.*—Numerous ingenious methods of measuring the viscosity of cytoplasm have been devised but the results obtained are not in agreement. The one conclusion which emerges most clearly from all of these studies is that the viscosity of the cytoplasm of living cells may vary greatly. It has also been found that not all of the cytoplasm in one given cell has the same viscosity at the same time. The surface layers of the cytoplasm are more viscous than the interior cytoplasm. In general cells that are physiologically active have cytoplasm of low viscosity while in dormant cells the cytoplasm

may be as stiff as a rigid gel. According to Seifriz (1936) fluid cytoplasm usually has a viscosity ranging from 800 (the consistency of glycerine) to 8000 (the viscosity of a thick sugar syrup) times that of pure water. On the other hand Heilbrunn (1928) considers active cytoplasm to have a viscosity only four or five times that of water. Probably most workers would consider the latter estimate a closer approximation to the facts than the former. The viscosity of the cytoplasm in active cells may change rapidly in response to mechanical injury or electric shock, to changes in temperature, differences in acidity, and exposure to various chemical compounds. Dehydration increases the viscosity of the cytoplasm and death of the cell results in a marked increase in viscosity.

4. *Immiscibility with Water.*—The cytoplasm of cells that are physiologically active is invariably composed predominantly of water, yet when the protoplasts are extruded from such cells into an aqueous medium they do not ordinarily mix with the water. The failure of cytoplasm to become dispersed through the water is largely, if not entirely, due to the presence of a surface membrane containing fat-like constituents which are insoluble in water. When this surface film is punctured a new membrane quickly forms across the broken surface. If substances are present that prevent the development of this surface film the cytoplasm of active cells readily disperses in the water.

5. *Gelation.*—Most of the cytoplasm of metabolically active cells exhibits the properties of a hydrophilic sol. In dormant or inactive cells, however, the cytoplasm apparently is often in a gel condition. No sharp line of division can be drawn between hydrophilic sols and gels and every gradation may exist in a given system between a well marked sol state and a well marked gel state (Chap. V). Every such gradation in physical condition may also exist in cytoplasm. It may range in physical state from a highly fluid sol through a viscous sol condition to a stiff elastic gel.

6. *Coagulability.*—The cytoplasmic system of most physiologically active cells is destroyed by temperatures of 60° C. or above. Death of plant cells by such temperatures is generally considered to result from a coagulation of some of the proteinaceous constituents of the protoplasm (Chap. XXXIII).

A number of other factors may bring about coagulation of the protoplasm, at least in the cells of certain species. Among these are certain electrolytes, electric currents, freezing, mechanical pressure, and certain wave lengths of radiant energy (especially ultraviolet radiations, X-rays, and radium radiations).

7. *Electrical Properties.*—Numerous attempts have been made to determine the isoelectric point of cytoplasm. It was soon discovered that cytoplasm does not have a definite isoelectric point but rather an isoelectric range.

This is doubtless due to the fact that cytoplasm is a complex sol composed of many different proteins, each one of which may possess an isoelectric point different from that of the others. Measurements of the cytoplasm of cells in the root tips of different plants showed the isoelectric range to be from pH 4.6 to pH 5.0 (Naylor, 1926). These values appear to be representative for cytoplasm in general.

Cytoplasm is usually on the alkaline side of its isoelectric range (see later discussion of the pH of the cytoplasm) and therefore we would expect to find that its constituent micelles are negatively charged. That this is true at least for the visible granules of the cytoplasm has been shown by Sen (1934). Cataphoretic migration of the granules in the cytoplasm of root hair cells and epidermal cells toward the anode was shown to occur, thus indicating that these particles carry a negative charge.

The proteins present in the nucleus apparently possess different isoelectric points from those of the cytoplasm. Furthermore, it is probable that some portions of the cytoplasm possess isoelectric points different from those of other parts of the cytoplasm in the same cell.

Cytoplasm contains dissolved electrolytes and would therefore be expected to conduct an electrical current. Brooks (1925) determined the electrical conductivity of the plasmodium of the myxomycete *Bicfeldia maxima* and found it to be equivalent to that of a 0.00145 N solution of NaCl, which is a relatively low value. The solution in the moss substrate on which the plasmodium was growing had a conductivity only about one-third as great as that of the plasmodium. Evidence was also found that the conductivity of the protoplasm varies according to the conductivity of the medium with which the cells are in contact.

8. *Streaming.*—In many cells the cytoplasm may be seen in active movement. In the simplest cases this movement consists of a rotation of the cytoplasm around the inner surface of the cell wall. Where cytoplasmic strands extend through the vacuole as in the case of cells of *Tradescantia* stamen hairs the circulation of the cytoplasm may become very complex. The fluid-like portion that is in motion is often bounded by thin layers of non-moving cytoplasm. The cytoplasm immediately adjacent to the cell wall and that which bounds the vacuole often does not move. The plastids and the visible granules are carried passively around the cell by the moving cytoplasm. The causes of cytoplasmic streaming (*cyclosis*) are unknown. It is accelerated by increases in temperature up to the point where injury appears and checked by low temperatures, ceasing at temperatures slightly above the freezing point. Cyclosis is also stopped in the absence of oxygen and by anaesthetics in relatively high concentrations. In dilute concentrations toxic substances such as

copper sulfate and narcotics accelerate the streaming movements. Light also appears to increase the rate of streaming under certain conditions.

The Physical Structure of Cytoplasm.—Any satisfactory explanation of the structure of cytoplasm must account not only for its physical properties and for its dynamic behavior, but must also explain how the innumerable diverse physical and chemical reactions characteristic of living cells can occur side by side in the same general medium. The highest magnifications reveal no evidence of any structural background in active cytoplasm, yet its complex activities suggest that it must possess an intricate structural organization. The marked imbibitional capacity of cytoplasm, its stability toward electrolytes, its electrical properties, its viscosity, its coagulability and its gel-forming capacity all suggest that it is to be classed with the hydrophilic colloids. All modern students of cytoplasm are agreed that it is a hydrophilic colloidal system of extreme complexity and variability.

As was pointed out in the preceding chapter elastic gels are generally considered to possess a submicroscopic structure of long interlacing fibrillar units which hold a liquid phase in their irregular interstices. The same structure has been suggested for some hydrophilic sols. There is some evidence that the structure of cytoplasm is similar. The cytoplasm in many living cells appears fibrous and long delicate strands may extend through the vacuole of the cell. Thread-like strands likewise appear in cytoplasm when it is being manipulated with very fine glass needles. The sudden changes in viscosity that living cytoplasm may undergo when disturbed, strongly suggest a fundamental structure similar to that of elastic gels. Furthermore, living protoplasm can itself flow slowly through small openings such as those in a fine sieve without injury, but cannot be forced through somewhat larger holes without serious injury or death. This behavior has been interpreted as evidence that a fiber-like structure exists in protoplasm; the fibers being able to slip through the holes when it is slowly streaming but being crushed and destroyed if a mechanical pressure is utilized to force the protoplasmic mass through the openings.

On the basis of this and other evidence living cytoplasm is presumed by some workers to have a skeletal structure of long submicroscopic fibrils. A fluid phase in which are suspended the visible droplets and granules is believed to be held in the interstices between the fibrils. Solutes may be presumed to be present in both phases. The whole system of fibrils and liquid is assumed to be dynamic and undergoing constant change, in which gel-like fibrils are rapidly transformed into sols and the sols form fibrils with equal rapidity. Such a structure will account satisfactorily for many of the properties of cytoplasm. For example, it offers an explanation for the rapid

changes in viscosity that are known to occur in cytoplasm, it accounts satisfactorily for its hydrophilic properties, and it suggests a possible solution to the perplexing problem of how so many diverse processes can go on in the same cytoplasm at the same time. Many of these reactions may occur at the interfaces between the various fibrillar units and the liquid constituents of the system. Since different fibrillar units are undoubtedly of different chemical composition, different chemical and physical reactions could take place, some at one interface and some at another. Furthermore, it is possible that some reactions occur within the liquid component and others within the more solid fibrillar units of the cytoplasm.

The Plastids.—All living cells of the higher plants contain prominent cytoplasmic bodies known as *plastids*. These structures are usually ellipsoidal in shape and are frequently conspicuous because of the presence of pigments. The color of the pigments in the plastids has been used as the basis for their classification but this system is highly artificial since a single plastid may be colorless, green, red or yellow at different periods of its existence.

In meristematic and embryonic cells the plastids first appear as very tiny granules in the cytoplasm that range in size down to the limit of visibility (Randolph, 1922). These granules gradually enlarge and differentiate until mature plastids are produced. In growing and in mature cells plastids frequently multiply by simple fission.

In the algae the chloroplasts exhibit a wide range of size and shape but in the higher plants they show a remarkable similarity. The mature chloroplast of the higher plants is typically a slightly flattened ovate spheroid with the longer axis ranging between 4 μ and 6 μ in length. The number of plastids is not constant in different cells nor in the same cell at different stages of its development.

The structure of chloroplasts has been more thoroughly investigated than that of other kinds of plastids. Most investigations indicate that the chloroplast consists basically of a proteinaceous matrix or *stroma* which is probably surrounded by a membrane. In at least some chloroplasts the chlorophyll apparently occurs in small disk-shaped granules called *grana* which are distributed throughout the stroma (Weier, 1938). The chlorophyll is apparently associated with both proteins and lipids. The yellow pigments carotene and the xanthophylls also occur in the chloroplasts.

The chromoplasts contain red or yellow pigments and are frequently very different in size and shape from the chloroplasts. Usually they are very slender spindle-shaped or needle-shaped bodies. They occur both singly and grouped in bundles. The irregular angular outline of these plastids contrasts

sharply with the regular curved surface of the chloroplasts. In some species, however, chloroplasts may develop red or yellow pigments and completely lose their green color. The red and yellow colors of some fruits, notably members of the *Solanaceae*, and some flowers are caused by chromoplasts.

The colorless leucoplasts are usually present in the cells of meristematic tissues in which they often represent juvenile stages in the development of chloroplasts and chromoplasts. In tissues not exposed to light they remain as colorless plastids and are the structures in which starch grains are formed.

Specialized plastids known as elaioplasts have been described as occurring in the cells of some species. These plastids appear to be centers of oil formation.

The Nucleus.—The nucleus is a conspicuous spheroidal body which is imbedded in the cytoplasm. In most plant cells the nucleus has a diameter which falls within a range of 5 μ to 25 μ. In the vascular plants there is usually only one nucleus to a cell, although in certain types of cells several may be present. The sieve tube elements are the only well known example of living cells in the higher plants in which no organized nucleus is present.

The nucleus is surrounded by a definite membrane and possesses a complicated internal structural organization. The larger portion of the volume of the nucleus is composed of a transparent, optically homogeneous sol or gel, the *nuclear sap*, which surrounds a system of delicate, anastomosing threads, the *reticulum*. The reticulum is not structurally homogeneous but contains irregular granules of material known as *chromatin*. Most nuclei also contain one or more *nucleoli*. Typically a nucleolus is a small spherical droplet of deeply staining protein and lipoidal material that is attached to the reticulum.

The hereditary factors which influence the development of the organism are known to reside in the nucleus of the cells. It is clear therefore that the nucleus must exert a controlling influence over the physiological activities of the cell. There is some evidence that the nucleus is concerned with the production of the enzymes which catalyze many, if not most, physiological processes. Very little is known, however, regarding the exact role of the nucleus in protoplasmic activities.

The Vacuole.—One of the most characteristic features of a mature plant cell is the presence of a large central vacuole filled with cell sap and entirely surrounded by the cytoplasm.

Merismatic cells in the tips of stems and roots usually contain numerous small vacuoles scattered throughout the cytoplasm. The shape of these vacuoles varies greatly and seems to be determined by the activity of the cytoplasm. In quiescent cytoplasm the small vacuoles are usually spherical but

when the cytoplasm is actively streaming they may assume many different forms. Cambium cells that are actively dividing may contain very large vacuoles (Bailey, 1930). In cambium initials the vacuoles, like those in the meristems of stem and root tips, are not uniform in size or shape, but may be rod-shaped, thread-like or globular. They may coalesce into a large single vacuole or they may divide into numerous smaller vacuoles. Mature cells, however, whether they arise from primary meristems or from the cambium cells typically contain one large central vacuole which arises by the increase in size and coalescence of the numerous smaller vacuoles usually present in the meristematic cells.

There is no general agreement as to the method by which vacuoles originate. Three possibilities are recognized: (1) They may arise by the division of pre-existing vacuoles, (2) they may originate *de novo* in the cytoplasm, and (3) they may develop from organized units of the cytoplasm. There is no convincing evidence, however, that the vacuoles of the cells of the vascular plants arise in any way except by the division of pre-existing vacuoles (Zirkle, 1937).

Among the various substances present as solutes in the vacuole are sugars, mineral salts, organic acids (oxalic acid, especially, seems to be of frequent occurrence), amino acids, amides, alkaloids, glycosides, flavones, and anthocyanins. Fats and related compounds often occur in finely emulsified form. Proteins, tannins, mucilages, lipids and other substances are commonly present in the colloidal state. Aleurone grains develop from specialized vacuoles of cells in storage tissues. Crystals of calcium oxalate are also of frequent occurrence in the vacuoles of mature cells.

Hydrogen Ion Concentration of Plant Cells.—The hydration and viscosity of the protoplasm, the permeability of the cytoplasmic membranes, the activity of enzymes, the chemical activity of various ions in the cell, and various other physiological processes and conditions are all influenced more or less by the hydrogen ion concentration of the protoplasm and the cell sap.

As soon as its significance was appreciated attempts were made to determine the hydrogen ion concentration of plant cells. Most of the earlier determinations were made on the juice pressed from plant tissues. Such a crude method provides only the roughest sort of an indication of the hydrogen ion concentrations in individual plant cells. The death of the cells and the mixing of the cell contents during the extraction process undoubtedly result in marked changes in hydrogen ion concentration. Such determinations are probably more nearly a measure of the pH of the cell sap than of the protoplasm. The values for expressed plant saps mostly fall within a range of pH 3.0 to pH 7.0

Direct measurements of the hydrogen ion concentration of the protoplasm and cell sap have been made by introducing indicator dyes directly into cells. By careful manipulation of a micropipette the dyes can be injected into the cytoplasm without penetrating into the vacuole or injected into the vacuole without penetrating into the cytoplasm. Only non-toxic dyes should be injected into living cytoplasm for pH determinations, else the results may be invalidated by injury or death of the cytoplasm. Dyes are considered to be non-toxic or essentially so if protoplasmic streaming continues in the same way as before the injection. In this manner it is possible to determine the reaction of the cell sap and that of the cytoplasm independently. Results of the micro-injection method when applied to the root hairs of the water plant *Limnobium spongia* indicated a pH value for the cytoplasm of 6.9 ± 0.2 (Chambers and Kerr, 1932). The cell sap of the same cells was found to be more acid, having a pH of 5.2 ± 0.2.

As indicated by the results cited above the pH of the cytoplasm and the cell sap of a plant cell may be very different. The pH of the cytoplasm of plant cells appears to be fairly constant, usually falling between 6.8 and 7.0 (Seifriz, 1936). The pH of the cell sap is usually lower than that of the cytoplasm, values between pH 5.2 and 6.2 seeming typical for most plant cells. The cell sap of some cells, however, shows a considerably more acid reaction than this, values as low as pH 0.9 being reported for species of Begonia (Smith and Quirk, 1926). On the other hand alkaline values have also been found in the vacuoles of some species (Haas, 1916). The cell sap appears to vary more widely in hydrogen ion concentration than the cytoplasm.

Buffer Action in Plant Cells.—Both the cytoplasm and cell sap of plant cells are usually buffered. The former, however, as its lesser variability in pH indicates, is usually more strongly buffered than the latter. All the actual information available regarding buffer action in plants is based, however, upon determinations made upon expressed plant saps.

The most important buffer systems in living organisms are those composed of a weak acid and one (or more) of its salts (Chap. II). The principal acids which are components of plant buffer systems are carbonic, phosphoric, citric, malic, tartaric, and oxalic. The first two of these acids are chemical progeny of compounds which enter the plant from its environment —carbon dioxide and the phosphates, respectively. The others are products of the metabolic activity of the cells. The principal base-forming elements present in the salts which may serve as components of buffer systems are sodium, potassium, calcium, and magnesium.

The buffer action of some types of plant cells is apparently due predomi-

nantly, if not almost entirely to one system. The buffering of lemon juice, for example, can be accounted for almost entirely in terms of the citrate buffer system. More commonly, however, a number of different buffer systems are present in a single cell.

Discussion Questions

1. How would you undertake to determine the number of plant cells in a leaf?
2. What are some of the distinctive differences between plant and animal cells?
3. List some of the phenomena characteristic of colloidal systems which occur in plant cells, citing specific examples.
4. Describe a living green plant cell from the physiological point of view. How will such a description differ from a morphological description of the same cell?
5. How would you undertake to determine whether the interior of a given plant cell was occupied by a central vacuole or largely by a mass of protoplasm?
6. Each one of a series of similar plant cells is immersed in a buffer solution of different pH, the solutions used covering a range of pH 4.0 to pH 8.0. What will be the effect on the pH of the cell sap of each cell? on the cytoplasm of each?

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CHAPTER VII

DIFFUSION

The chemical elements which constitute the bulk of the body of any plant are among the commonest ones on the surface of the earth. They occur in the environment of plants as relatively simple inorganic compounds, and enter plants in the form of such compounds. From the relatively small number of compounds which enter it from its environment the green plant fabricates the numerous complex organic compounds which are essential to its continued existence as a living system.

The movement of substances into a plant from its surroundings is accomplished principally through the agency of one form or another of the process known as *diffusion*. Substances enter a plant partly through its aerial organs, and partly by way of its root system. From the atmosphere carbon dioxide and oxygen gases diffuse into plants, principally through the stomates. From the soil, water and the cations and anions of simple inorganic salts pass into plants by processes which are basically diffusion phenomena, although the entrance of both water and mineral salts is complicated by other factors.

Similarly the loss of substances from a plant into its environment is accomplished principally by diffusion. Large quantities of water-vapor pass out of leaves and other aerial organs of plants into the atmosphere. At times oxygen gas and at times carbon dioxide gas diffuse into the atmosphere. Certain volatile compounds also escape from the aerial organs of many plants by diffusion. Similarly the roots lose carbon dioxide and other compounds into the soil by diffusion.

Likewise some of the movement of substances from one part of a plant to another is accomplished by diffusion. This is true both of the gases which move through the intercellular spaces and the water and solutes which move within the cells. However, much of the translocation of materials from one plant organ to another is accomplished by more complicated mechanisms than diffusion. Within any living plant cell diffusion of substances from one part of a cell to another is also continually in progress.

In brief, there are few if any of the physiological processes occurring in plants which do not directly or indirectly involve diffusion phenomena.

Diffusion of Gases.—If a small vial of bromine gas is broken under a bell jar which has previously been evacuated of air the entire jar quickly becomes filled with the brownish vapor of bromine. The distribution of the bromine gas throughout the bell jar has been accomplished by the kinetic activity of the bromine molecules, and is a simple example of the process of diffusion. If the vial of bromine be broken under a bell jar which has not been evacuated of air, the time required for the bromine gas to completely occupy the bell jar will be longer than when diffusion of the gas occurs into a vacuum. Under such conditions the freedom of movement of the bromine molecules is greatly impeded by the presence of molecules of the gases of the air, and the diffusion process is retarded. If the pressure of the air within the bell jar be increased to two atmospheres (which is equivalent to doubling the concentration of all the gases in the jar), the rate of diffusion of the bromine gas through the air would be still less than when the jar was occupied by air at atmospheric pressure.

Many other simple examples of the diffusion of gases might be cited. If a bottle of ammonia, ether, peppermint oil, or of any other readily volatile substance with a characteristic odor be opened indoors, within a very short time the distinctive odor of that substance can be detected in all parts of the room. Such a dispersal of gas molecules is accomplished at least in part by diffusion, although air currents often assist in speeding up such a distribution of molecules. Except in the rare case of diffusion into a vacuum diffusing molecules move between the molecules of other substances.

The following somewhat fanciful analogy may aid in a visualization of the kinetics of the diffusion process in gases. Suppose two adjoining rooms to be connected by a closed double door. Imagine also that one of these rooms contains a large number of tennis balls travelling in various directions along straight pathways at different rates of speed. The average distances between the tennis balls are supposed to be relatively great in proportion to their diameters. Each tennis ball represents a molecule. The individual balls will be constantly bumping into each other and into the walls of the room. Because of the large number of balls present innumerable collisions will occur every second. Each time a ball strikes a wall of the room it will bounce off along a different linear pathway. Similarly, whenever two balls collide, each will be deflected out of its course along a different route, to which it will hold undeviatingly until it is again deflected from its path by another collision. The course of each ball will thus be a zigzag progression through space, each short segment of its path being terminated by a collision which changes its direction. All of the balls do not move at the same speed at any given time, but the speed of each fluctuates from moment to moment as a result of the

numerous collisions in which it participates. The average speed of the entire group will remain constant, however, as long as the temperature remains unchanged. As a result of this haphazard mutual buffeting, the tennis balls will remain essentially equally distributed throughout the room.

Suppose now that the double doors connecting the two rooms are thrown open. As a result of their haphazard movement some of the balls close to the door will pass into the empty room. The first ones to do this will travel without interruption until they bump into one of the walls, as their rate of progression will not be impeded by collisions with other molecules. When they hit against one of the walls of the room they will bounce back into its interior along a new pathway. As more and more of the balls pass into the originally empty room their rate of progress becomes slower since the greater their concentration in the room, the greater the number of collisions per unit of time. As soon as any appreciable number of tennis balls has invaded the empty room, as a result of their random movements, some will pass back through the doorway into the room which originally contained all of them. As long, however, as the concentration (number per unit volume) of the tennis balls is greater in one room than in the other their random movements will result in more passing through the door into the room in which their lesser concentration prevails, than in the opposite direction.

In a relatively short time the concentration of tennis balls will have become equal in both rooms. In other words they have "diffused" from one room into the other. After equality of concentration has been established the number of balls passing through the door in one direction in any interval of time will be exactly equal to the number moving in the opposite direction. When this condition of dynamic equilibrium is attained, diffusion, in the sense the word will be used in this discussion, is no longer occurring.

If, in the hypothetical illustration just described, one room were filled with white tennis balls, and the other with red tennis balls, diffusion would occur simultaneously in both directions. The red balls would "diffuse" toward the room in which their initial concentration was zero while the white balls would "diffuse" in the opposite direction. At equilibrium the concentration of the white balls would be equal throughout the two rooms, and this would likewise be true of the red balls.

Even after a dynamic equilibrium has been attained in any system haphazard kinetic activity of the molecules continues. This is sometimes referred to as diffusion, but will not be so considered in this book. The term diffusion will be used only to characterize situations in which there is gain in the number of molecules of a certain kind in one part of a system at the expense of other parts. According to this concept diffusion can only occur when

the concentration of the diffusing substance is not uniform throughout the system, and the process can continue only as long as differences of concentration are maintained.

Diffusion dependent upon the kinetic energy of molecular motion and thus resulting solely from differences in concentration is often called "simple diffusion" in order to distinguish it from more complex types of diffusion phenomena in which other forces come into play. Examples of such more complex phenomena will be encountered in some of the following chapters.

The phenomenon of diffusion is exhibited by the molecules of liquids, solutes, and even solids, as well as by those of gases. Diffusion of ions also occurs, and even colloidal particles diffuse, but only at very slow rates (Chap. V).

Diffusion phenomena should be clearly distinguished from *mass movements,* in which the moving units are not single molecules, but more or less extensive assemblages of molecules. Winds and air currents generally are examples of mass movements of gas molecules. The draft of warm air ascending a chimney is another. All of the phenomena just listed, and many others, are due primarily to differences in the density of the gases in various parts of a system. Heavy gases are more strongly attracted by gravitational forces than light gases. Hence, as in a chimney, cold (relatively heavy) air, displaces hot (relatively light) air, forcing the latter to rise. Similar phenomena on a grand scale are the principal cause of winds and air currents. They are representative of the physical process called *convection.* Such phenomena also occur in liquids. Mass movements of gases and liquids can also be caused in many other ways.

Diffusion Pressure.—That diffusing gases sometimes result in the development of measurable pressures can be readily shown by certain simple experiments. In Fig. 17 is depicted an apparatus which can be used to illustrate a number of aspects of the phenomenon of diffusion of gases. This apparatus consists essentially of a vertically arranged glass tube, to the upper end of which is attached, by means of rubber stopper, a cylindrically shaped porous clay cup. This cylinder is hollow and its thick walls are pierced by numerous minute capillaries. These pores are fine enough to prevent mass movement of gases at any appreciable rate, but the porous clay is not a membrane in the usual sense of the word. The lower end of the vertical glass tube dips in some colored water. If such a porous clay cylinder, containing air, be enclosed within a bottle containing hydrogen gas a rapid bubbling of gas will occur from the lower end of the tube through the dye solution into which it dips. When the bottle is removed a rapid and sudden rise of liquid up the glass tube will ensue. This is followed by a slow subsidence in the

level of the liquid in the glass tube, until it falls to that of the water in the beaker.

The explanation of this sequence of events is as follows. For reasons which are discussed later the *rate* of diffusion of hydrogen is greater than that of nitrogen or oxygen under comparable conditions. When the porous clay cup is first enclosed within the bottle of hydrogen, rapid diffusion of hydrogen gas occurs through the pores of the cup. This raises the total gas pressure within the cup, since outward diffusion of oxygen and nitrogen occurs at a much slower rate. Hydrogen gas diffuses into the cup in spite of the fact that this results in a greater total pressure inside the cup than in the surrounding atmosphere. The *direction* of the diffusion of the hydrogen gas is controlled entirely by its own differences in concentration, and is unaffected by the presence of other gases. The greater gas pressure inside the cup results in the outward bubbling of a mixture of all three gases at the lower end of the vertical glass tube. When the bottle is removed from around the porous clay cup, the direction of the diffusion of hydrogen is reversed, since the concentration of hydrogen gas is now greater inside of the cup. Since, while enclosed in the bottle of hydrogen, some of the nitrogen and oxygen diffused out of the cup and some was lost by the bubbling of gases from the lower end of the glass tube, this rapid outward diffusion of hydrogen results temporarily in a lower total gas pressure inside the cup than that originally present. Hence the solution rises rapidly in the glass tube. Finally, nitrogen and oxygen diffuse slowly inward, because of the reduced concentration of these gases inside of the cup, and the liquid slowly falls in the glass tube to its original level.

That the process of diffusion may result in the development of pressure also can be shown very strikingly by means of another simple experiment. If a pure rubber balloon containing only a little air be suspended in a closed bottle of carbon dioxide gas it will gradually become distended. Rubber membranes are quite readily permeable to the molecules of carbon dioxide but virtually impermeable to those of oxygen, nitrogen, or other gases of the atmosphere. Since, temperature remaining constant, the pressure exerted by any gas is directly proportional to its concentration (number of molecules per unit volume), it follows that the diffusion of the molecules of gases may be interpreted in terms of the differences in the *partial pressure* (Chap. II) exerted by that gas in different parts of a system. Because of the greater partial pressure of carbon dioxide in the surrounding atmosphere, diffusion of this gas through the walls of the balloon continues until the partial pressure of the carbon dioxide is the same on the two sides of the rubber membrane. Since the initial partial pressure of carbon dioxide inside the balloon was

virtually zero, and that in the bottle equivalent to one atmosphere, the inward diffusion of carbon dioxide gas results in a considerable distension of the balloon.

In the analysis of diffusion phenomena it is often clarifying to speak of the partial pressure of a gas as its *diffusion pressure.* Solutes and liquids

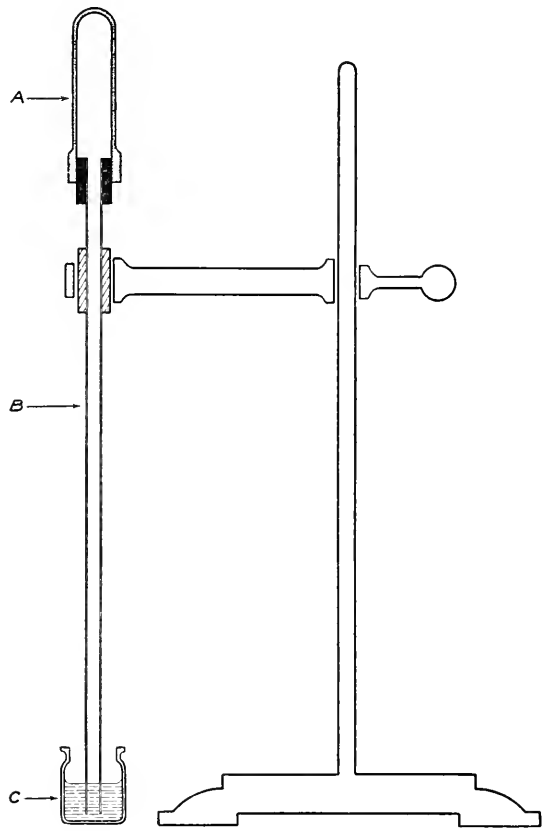

FIG. 17. Apparatus for demonstrating the development of pressure during diffusion of gases. (*A*) porous clay cup, (*B*) glass tube, (*C*) vessel of colored water.

may also be considered to possess a diffusion pressure, although the existence of such a physical quantity is less evident in such systems than in gases. Hence the concept that diffusion is the movement of the molecules of a substance from a region of its greater to a region of its lower diffusion pressure is often more satisfactory than the interpretation of diffusion phenomena in terms of concentration differences.

Principle of Independent Diffusion.—In the first experiment described in the preceding section it was shown that while one gas (hydrogen) was diffusing in an inward direction through the pores of a clay cylinder, other gases (oxygen and nitrogen) were simultaneously diffusing in an outward direction through the same pores. This exemplifies one of the most important principles governing diffusion phenomena, namely that the *direction* in which any substance will diffuse is controlled entirely by its own differences in diffusion pressure, and is not influenced by either the direction or rate of diffusion of other substances in the same system. Hence, in any given system, as for example, two adjacent plant cells, a number of substances may be diffusing in one direction across the intervening membranes, while simultaneously other compounds may be diffusing in the opposite direction across the same membranes. Each one of these individual substances will diffuse in the direction determined by its own differences in diffusion pressure, and at a speed which is determined by the factors which are influencing the diffusion of that particular substance.

Factors Influencing the Rate of Diffusion of Gases.—1. *Density of the Gas.*—Different gases diffuse at different rates even when influenced by the same set of environmental factors. Hydrogen, for example, diffuses more rapidly than any other gas. The same Thomas Graham who conducted some of the earliest studies on the properties of colloidal systems also was one of the first investigators to study quantitatively the phenomenon of gaseous diffusion. He discovered the principle, often called "Graham's Law of Diffusion," that the relative speeds of diffusion of different gases are inversely proportional to the square roots of their relative densities. By relative density is meant the weight of a given volume of gas as compared with the weight of the same volume of hydrogen.[1] The relative density of oxygen is 16. Hence the rate of diffusion of hydrogen is proportional to $\frac{1}{\sqrt{1}}$, while that of oxygen is proportional to $\frac{1}{\sqrt{16}}$. Hydrogen gas will therefore diffuse four times as rapidly as oxygen gas under the same conditions of temperature and pressure. The relative density of carbon dioxide is 22; hence by similar reasoning it is apparent that hydrogen gas will diffuse nearly five times as rapidly as carbon dioxide gas.

[1] Since a molar weight of any gas occupies a volume of 22.4 liters at standard conditions, molar weights of gases are in themselves a measure of the relative density of gases. Since the molar weight of hydrogen (H_2) is 2.016, the relative density of any gas on the basis H = 1, is equal to its molar weight divided by 2.016 (usually rounded off to 2).

2. *Temperature.*—Increase in temperature increases the speed of diffusion. This is due, at least in part, to the correlated increase in the kinetic activity of the molecules of the diffusing substance. Actual measurements of the temperature coefficient [2] of diffusion generally yield values between 1.2 and 1.3. Such values are characteristic of any purely physical process such as diffusion.

3. *Diffusion Gradient.*—The fact that the *direction* of the diffusion of gases is from a region of their greater diffusion pressure to a region of their lesser diffusion pressure has already been emphasized. The *speed* of diffusion is also influenced by differences in diffusion pressure. In general the greater the difference in diffusion pressures between the two regions, the more rapidly diffusion will occur. The rate of diffusion is influenced, however, not only by the difference in diffusion pressures, but also by the distance through which the diffusing molecules must travel. These two factors are components of what may be called the *diffusion pressure gradient* or *concentration gradient.* This concept may be clarified by a specific illustration such as that depicted in Fig. 18. In both parts, *A* and *B,* of this diagram, oxygen is represented as diffusing from a region in which its diffusion pressure is maintained at 760 mm. Hg (atmospheric pressure) into a region in which its diffusion pressure is just half as great. The length of the connecting tube is, however, just twice as great in *A* as in *B*. Under the conditions postulated the rate of diffusion from the region of greater diffusion pressure to the other will be just twice as rapid in *B* as in *A*. The diffusion pressure gradient is equivalent to the difference in the diffusion pressures between the delivering and receiving ends of the diffusion system divided by the length of the distance between. The greater or "steeper" this gradient the more rapidly diffusion will occur. The steeper the gradient, the more rapid the change in diffusion pressure per unit of length along the axis of the diffusion gradient. The steepness of a diffusion pressure gradient may be changed by varying either factor, the difference in diffusion pressures, or the length of the gradient.

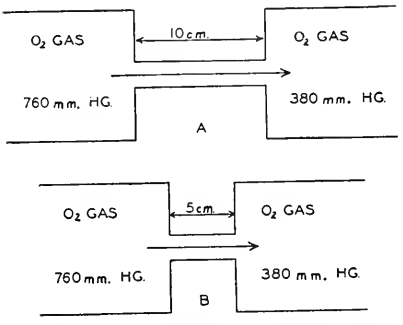

FIG. 18. Diagram illustrating that the length of a diffusion gradient is a factor influencing its steepness.

[2] The temperature coefficient (Q_{10}) of any process, physical, chemical, or physiological, is defined as the number of times that the rate of the process increases with a 10° C. rise in temperature. If the rate of the process is doubled, the temperature coefficient is 2, etc.

4. *Concentration of the Medium through Which Diffusion Occurs.*—In general the more concentrated the medium, *i.e.* the more molecules per unit volume in the medium through which the diffusing molecules must pass, the slower the rate of diffusion. Bromine gas, as shown earlier in this chapter, diffuses more rapidly through a vacuum than through air.

Diffusion of Solutes.—The molecules or ions of a solute possess sufficient kinetic energy to move from place to place within the limits of a solution. The simplest method of demonstrating the diffusion of a solute is to introduce a crystal of copper sulfate, or some other compound which is colored when in solution, into the bottom of a tall glass cylinder filled with water. The cylinder should then be placed in an environment of equable temperature where it will be free from disturbance. The diffusion of the molecules or the ions which pass into solution in the water can be followed by the slow change in color of the water. One of the most striking facts illustrated in such experiments is the extremely slow rate of diffusion of solutes through water. This is partly due to the fact that in such an experiment the steepness of the diffusion gradient decreases with time, but principally to the fact that the densely packed molecules of the liquid enormously impede the diffusion of the dissolved molecules or ions.

The true rate of diffusion of solutes is probably even less than the rates indicated in such experiments, since in all such set-ups convection currents may develop in the water and aid in the distribution of the solute throughout the body of the liquid. The diffusion of solutes is often demonstrated by employing a gel rather than a liquid as the medium into which diffusion occurs (Chap. V). Such a technique avoids errors introduced by the development of convection currents.

The *direction* of the diffusion of any solute occurs in accordance with its own differences in diffusion pressure, regardless of the rate or direction of diffusion of other solutes in the same system. The *rate* of diffusion of solute particles is governed by principles essentially similar to those which control the rate of diffusion of gases and is controlled by the following factors:

1. *Size and Mass of the Diffusing Particle.*—Small molecules or ions diffuse more rapidly than large ones. A hydrogen ion, for example, diffuses many times more rapidly than a glucose molecule. Similarly, highly hydrated ions diffuse more slowly than those which have fewer water molecules bound to them, since the association of water of hydration with a molecule or ion in effect increases its size. The mass of the particle will also be a factor influencing the speed of its diffusion. As between two particles of the same size, but different masses, the heavier particle will diffuse more slowly.

2. *Temperature.*—The kinetic activity, and hence the rate of diffusion of solute molecules, increases with increase in temperature.

3. *Diffusion Gradient.*—The steeper the diffusion gradient, the more rapidly solute particles diffuse.

4. *Solubility.*—In general, the more soluble a substance is in a liquid, the more rapidly it will diffuse through that liquid. This influence of solubility upon diffusion rates can be interpreted principally in terms of its effect upon the diffusion gradient, since obviously steeper gradients can be built up if the solute is very soluble in the liquid than if it is only slightly soluble.

Discussion Questions

1. Why, in an experiment in which copper sulfate crystals are put in the bottom of a tall cylinder, does the steepness of the diffusion gradient decrease with time?
2. If, in the rubber balloon experiment described in the text, the initial pressure of carbon dioxide outside the balloon is 1 atmos. what will be the approximate pressure of the carbon dioxide inside the balloon at equilibrium? outside the balloon?
3. Two glass containers A and B are connected by a short length of small bore glass tubing which is closed with a stopcock. Describe what will happen when the stopcock is opened under the following conditions: (a) A contains CO_2 at 1 atmos. pressure and B contains H_2 at 1 atmos. pressure. (b) A contains 1 volume of CO_2; B contains 1½ volumes of CO_2, but the pressure in A equals that in B because of its higher temperature. (c) A and B each contain CO_2 at 1 atmos. pressure when the stopcock is opened. The temperature in A is then lowered 10° C. (d) A contains one volume of O_2 and 2 volumes of CO_2; B contains 2 volumes of O_2 and one volume of CO_2.
4. How can the CO_2 molecules exert a pressure against the inside walls of the rubber balloon in the experiment described in the text when rubber is permeable to CO_2?
5. The cation of a mineral salt often accumulates in the vacuole of a plant cell in greater concentration than it was present in the external solution. Is it possible to account for such a phenomenon in terms of simple diffusion?
6. If a porous clay cup arranged as in Fig. 17 is surrounded with a bottle containing pure CO_2 the water in the glass tube will rise. If the cylinder is first dipped in water and then surrounded by a bottle containing CO_2, gas will slowly bubble out of the lower end of the vertical tube. Explain.

Suggested for Collateral Reading

Mellor, J. W. *Modern inorganic chemistry.* Longmans, Green and Co. London. 1925.

CHAPTER VIII

OSMOSIS AND OSMOTIC PRESSURE

Although the freedom of movement of the molecules of liquids is to some extent restrained by internal cohesive forces, they also possess kinetic activity. Like gases and solutes, therefore, liquids exhibit diffusion phenomena. If, for example, water is brought into contact with another liquid such as ether with which it is only slightly miscible, a slow diffusion of water molecules into the ether will occur. Simultaneously a slow diffusion of the molecules of the ether will take place into the water. Such diffusion will continue until the two liquids are mutually saturated.

Osmosis.—This is by far the most familiar process involving the diffusion of liquids. An understanding of the dynamics of this process, and of the significance of the physical quantity termed osmotic pressure is essential to an interpretation of the water relations of plant cells and tissues.

Let us first consider an experiment arranged as in Fig. 19. A sac-like membrane of collodion is nearly filled with a strong sucrose solution and immersed in a beaker of water. The top of the sac is tightly plugged with a rubber stopper. The collodion membrane is so prepared that it is permeable to water, but impermeable or practically so to sucrose; in other words it is *differentially permeable* (Chap. X). It is also slightly elastic.

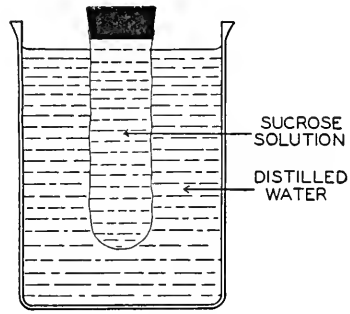

FIG. 19. Apparatus for the demonstration of osmosis through a collodion membrane.

After a short time the originally limp sac becomes rigidly distended. This is due to the movement of water through the collodion membrane into the interior of the sac. Such a diffusion of water is an example of *osmosis*. The pressure developed as a result of the entrance of water is exerted against the inside wall of the membrane. This pressure is counterbalanced by an equal and oppositely directed pressure exerted upon the solution by the distended wall. If the membrane is virtually impermeable to

the solute eventually the entire system will come to equilibrium after which there is no further increase in the volume of water inside of the membrane.

When the movement of the solvent is referred to in discussing osmosis it is always the net movement which is meant. Solvent molecules will always be moving across the membrane in both directions, but except when an equilibrium has been attained, more molecules will move per unit of time in one direction than in the other. As will be shown in the later discussion this net movement is always from the region of the greater diffusion pressure of the solvent molecules to the region of their lesser diffusion pressure. The maintenance of the diffusion gradient of the liquid in most osmotic phenomena is due to the relative impermeability of the membrane to the molecules of solutes. It is the presence of this differentially permeable membrane which gives to osmosis its distinctive aspect as compared with other diffusion processes. If the membrane were permeable to the solute particles, they would diffuse in one direction, while the molecules of the solvent were diffusing in the other; this would be another example of the principle of independent diffusion.

Terminology.—The conditions under which osmosis can occur are so varied that it is impossible to characterize the process in terms of a simple definition. The following statement will, however, apply to most cases: Osmosis is the diffusion of a liquid through a differentially permeable membrane into a solution in which the solvent is that same liquid or into another liquid with which it is miscible. In living organisms water is the only important liquid which moves from cell to cell by osmosis, hence the further discussion of this process will be in terms of water and aqueous solutions.

The attainment of a satisfactory comprehension of osmosis and osmotic pressure has been delayed both by confusion in theoretical interpretations as well as by lack of a consistent terminology. Many obscurities have resulted from the application of the term osmosis to the movement of solutes through membranes. The use of the term in this sense has not only led to many misunderstandings, but also to positive errors and misinterpretations of data. The movement of solutes through a membrane is a diffusion process; it may be simple diffusion, or it may be complicated by a number of factors which do not enter the phenomenon of simple diffusion. Both from the historical and theoretical standpoint it seems desirable that the term osmosis be restricted to the sense in which it is used in this discussion.

Osmotic pressure may be defined as the *maximum* pressure which can be developed in a solution when separated from pure water by a rigid membrane permeable only to water. In order to determine the osmotic pressure of a solution it would be necessary to enclose it in a rigid membrane permeable only to water, to immerse this membrane in pure water, and to exert just

enough pressure on the solution (by means of a leak-proof piston, for example) to prevent any increase in its volume due to the entrance of water. The osmotic pressure of the solution is quantitatively equal to the imposed pressure. If the latter is 15 atmos., then the osmotic pressure of the solution is 15 atmos., etc.[1]

It is a common practice to speak of solutions as possessing an osmotic pressure whether they are under such conditions that a pressure can develop within them or not. A weight molar solution of sucrose contained in a bottle may therefore be spoken of as possessing an osmotic pressure of 27 atmos. at 25° C. (Table 13). In other words the term osmotic pressure is commonly used to denote the *potential* maximum pressure which would develop in a solution were it placed under the necessary conditions. Its use will be confined to this sense in this book.[2]

The term osmotic pressure is also often used as a designation for the *actual* pressures developed as a result of osmosis, a procedure which leads to confusion. Actual pressures developed during osmosis are seldom equal to the osmotic pressure as defined above. If a solution having an osmotic pressure of 12 atmos. be enclosed in a stoppered membrane permeable only to water which is in turn immersed in a solution having an osmotic pressure of 8 atmos., water will diffuse inwards until at equilibrium the actual pressure developed in the internal solution will be (disregarding any minor effect due to dilution) 4 atmos. (see later). Its osmotic pressure will still be nearly 12 atmos., however. Even if the external liquid is pure water, the actual pressure developed in the internal solution would not be equal to its original osmotic pressure unless the membrane is completely inelastic. Inward diffusion of water results in a dilution of the solution which, as we will see later, always results in a decrease in its osmotic pressure. To avoid the inevitable confusion caused by use of the same term in a dual sense we might distinguish between the *potential* (*i.e.* maximum) osmotic pressure and the *actual* osmotic pressure of a solution. In this discussion however, we shall employ the term *turgor pressure* to refer to the *actual* pressure developed as a result of osmosis. This term has long been employed as a name for the actual

[1] Osmotic pressure is sometimes defined as the pressure which must be imposed on a solution to prevent any increase in its volume under the conditions specified above, but this usage is particularly unsatisfactory in the interpretation of biological phenomena. While the magnitudes of such an imposed pressure and the osmotic pressure are mathematically equal, physically they are two entirely different quantities.

[2] The osmotic pressure of a solution has also been variously called the *osmotic potential, osmotic power, osmotic value*, and *osmotic concentration* of a solution. Such terms are frequently encountered in the literature of plant physiology.

pressures developed in plant cells. Since these are largely of osmotic origin the name turgor pressure seems equally appropriate for physical systems. Actually the turgor pressure developed in a solution enclosed within a membrane may be increasing while its osmotic pressure is decreasing, as shown in the last example mentioned above. This indicates clearly that each of these terms—osmotic pressure and turgor pressure—refers to a different physical quantity. In an osmometer set up as just described the maximum turgor pressure which could be developed will never exceed the osmotic pressure.

The Mechanism of Osmosis.—Like a gas, water or any other liquid may be considered to possess a diffusion pressure (Haldane, 1918). The only two factors which influence the diffusion pressure of a pure liquid are pressure and temperature. The imposition of pressure from an external source will raise the diffusion pressure of a liquid. For example, if a pressure of 10 atmos. be exerted upon water in a closed vessel by means of a leakproof piston, the diffusion pressure of that water will be increased by 10 atmos. Under certain conditions "negative pressures" or tensions may develop in liquids. These result in a reduction in the diffusion pressure of the liquid in an amount equal to the magnitude of the tension.

Although the diffusion pressure of water is also influenced by temperature no interpretation of the influence of this factor will be undertaken. In order to simplify the following discussion it will be consistently assumed that all parts of every osmotic system considered are at the same temperature.

However, when a substance is dissolved in water the diffusion pressure of the water in the resulting solution is decreased as compared with that of pure water at the same temperature and pressure. This diminution in diffusion pressure is proportional, within a wide range of solution concentrations, to the number of solute particles present in a given volume (*i.e.* a given number of molecules) of the solvent. In a solution, therefore, the diffusion pressure of the solvent may be influenced by the three factors of (1) temperature, (2) pressure, and (3) the ratio of solute particles to solvent molecules.

When a solution is confined within a membrane permeable only to water and that membrane is immersed in water there will be a net movement of water through the membrane into the solution, because of the excess diffusion pressure of the water on the water side of the membrane. The passage of water through the membrane results in the development of a turgor pressure on the solution side of the membrane. Since the maximum possible pressure (=osmotic pressure) which can develop in the solution is equal to the excess of the diffusion pressure of pure water over the diffusion pressure of the water in the solution, the following relation holds, providing all parts of

the system are at the same temperature and under the same external pressure:

Osmotic pressure = Diffusion pressure of pure water − Diffusion pressure of water in the solution.

In other words the osmotic pressure of a solution is a measure of what may be called the *diffusion pressure deficit* of the water in that solution. By this term, as applied to any given solution, is meant the amount (usually expressed in atmospheres) by which the diffusion pressure of the solvent in that solution is less than the diffusion pressure of the pure solvent when the latter is at the same temperature and under atmospheric pressure. For example, if a solution has an osmotic pressure of 10 atmos., this signifies that the *diffusion pressure* of the water in that solution is just 10 atmos. less than the diffusion pressure of pure water under atmospheric pressure at the same temperature; in other words its *diffusion pressure deficit* is 10 atmos. The osmotic pressure of a solution is a measure of the diffusion pressure deficit of the water in that solution *only* when the solution is not under a pressure or a tension (except, of course, atmospheric pressure) since these factors also affect the diffusion pressure of water. In plant cells, as we shall see later, pressures and tensions must constantly be taken into account in evaluating the diffusion pressure deficit of the water in the cell sap.

When two aqueous solutions, both initially subjected only to atmospheric pressure, are separated by a membrane permeable only to water, diffusion of water will take place towards the solution of greater osmotic pressure. For example, if solution A with an osmotic pressure of 20 atmos. be enclosed in a stoppered membrane permeable only to water which is immersed in solution B with an osmotic pressure of 12 atmos., water will diffuse inwards, *i.e.* towards solution A. The diffusion pressure deficit of the internal solution A is 20 atmos.; that of the external solution B, 12 atmos. Water always moves in any osmotic system towards the region of its lesser diffusion pressure, or in other words towards the region of its greater diffusion pressure deficit. In this particular example, disregarding dilution, the internal solution will exert a *turgor pressure* of 8 atmos. at equilibrium. Correspondingly the membrane will exert a *wall pressure* of 8 atmos. against the enclosed solution. Since subjection to a pressure raises the diffusion pressure of water by the amount of the imposed pressure, the diffusion pressure deficit of the water in the internal solution at equilibrium will not be 20 atmos., but 12 atmos. In other words, at equilibrium, the diffusion pressure deficit of the water will be equal on both sides of the membrane.

In the preceding discussion it has been tacitly assumed that water diffuses through the membrane during osmosis in the liquid state. Many investigators consider, however, that actual diffusion of water across the membrane occurs

in the form of water-vapor. Callendar (1908) who first proposed such a "vapor pressure theory" of osmosis, assumed the existence in differentially permeable membranes of minute capillary pores too small to be wetted by liquids, but freely permeable to molecules in the vapor state. Warrick and Mack (1933) have described an example of an inorganic membrane through which water passes in the vapor state, but it is impossible to say whether or not this is also true of the differentially permeable membranes of plant cells.

The essential concepts in the vapor pressure theory of osmosis can be presented in terms of two simple experiments. Figure 20, *A* represents an inverted U tube, one arm (x) of which is partially filled with a sucrose solution while the other arm (y) is filled to the same level with pure water. The space above the liquids contains only air plus the water-vapor which enters it from the liquids. The air acts as a membrane which is permeable to water (vapor), but not to sucrose molecules. The presence of the sucrose molecules decreases both the diffusion pressure and the vapor pressure of the water in the solution. In fact, *increase* in the diffusion pressure deficit (osmotic pressure) of a solvent caused by the introduction of a solute is accompanied by a proportionate *decrease* in its vapor pressure. As a result the vapor pressure just above the layer of pure water will be greater than that above the layer of the solution, and a gradient of vapor pressures will be established, decreasing in magnitude from the water to the solution. Water will therefore move from the region of high vapor pressure to the region of low vapor pressure, and the solution will steadily gain in volume at the expense of the pure water. Ultimately all of the water will be transferred to the sucrose solution. The transfer of water from one arm to the other has resulted from the difference in vapor pressures of the two liquids and is therefore directly correlated with their differences in diffusion pressures.

If this experiment were modified as illustrated in Fig. 20, *B*, where the sucrose solution in x is separated from the water in y by a rigid membrane permeable only to water, and evaporation prevented from the open arms of the U-tube by thin layers of oil, there will be a net movement of water from y to x. There will be an increase in the volume of the solution in x, and a decrease in the volume of the water in y. If we assume that the water moves through the membrane in the form of vapor there is no essential difference between this phenomenon and the one first described.

In the foregoing discussion the effect of temperature on the osmotic movement of water has been disregarded. If different parts of an osmotic system are maintained at different temperatures this will have an effect on the osmotic movement of water. However unless the difference in temperature on the

two sides of the membrane is very considerable the effects of this factor on osmosis are slight, and usually can be disregarded.

The attainment of an osmotic equilibrium between two solutions, or between a solution and water does not necessarily imply an equalization of solute concentration, nor of pressure, nor of temperature. It does imply, however, an equalization of diffusion pressures and an examination of the

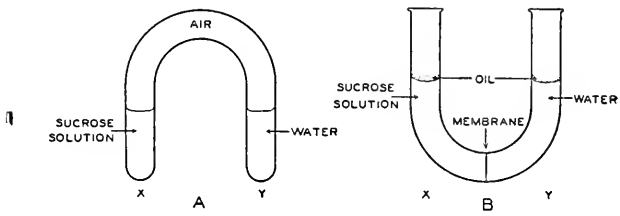

FIG. 20. Diagrams illustrating discussion of the vapor pressure theory of osmosis.

circumstances is necessary to determine the exact mechanism by which they have been brought into equilibrium in any given system.

Analogies between Osmotic Pressure and Gas Pressure.—In Tables 13, 14, and 15 are summarized data which illustrate the relations between osmotic pressure and (1) concentration of the solution, (2) temperature, and (3) kind of solute.

TABLE 13—THE RELATION BETWEEN THE WEIGHT MOLAR CONCENTRATIONS OF SUCROSE SOLUTIONS AND THEIR OSMOTIC PRESSURES AT 25° C. (DATA OF MORSE, 1914)

Weight molar concentration	Osmotic pressure (experimentally determined)	Calculated "gas pressures" of sucrose (van't Hoff hypothesis)	Ratio osmotic to gas pressures
0.1	2.634 atmos.	2.431 atmos.	1.084
0.2	5.148	4.862	1.059
0.3	7.729	7.292	1.060
0.4	10.296	9.723	1.059
0.5	12.943	12.154	1.065
0.6	15.625	14.585	1.071
0.7	18.435	17.015	1.083
0.8	21.254	19.446	1.093
0.9	24.126	21.877	1.102
1.0	27.053	24.308	1.113

Within this range of concentrations, as shown by the data in this table, the osmotic pressures of sucrose solutions are closely proportional to their

weight molar concentrations. The significance of the last two columns in this table will be discussed later.

TABLE 14—THE RELATION BETWEEN TEMPERATURE AND THE OSMOTIC PRESSURES OF WEIGHT MOLAR SUCROSE SOLUTIONS (DATA OF MORSE, 1914)

Temperature ° C.	Osmotic pressure (experimentally determined)	Calculated "gas pressures" of sucrose (van't Hoff hypothesis)	Ratio osmotic to gas pressure
0	24.826 atmos.	22.265 atmos.	1.115
10	25.693	23.082	1.113
20	26.638	23.899	1.115
30	27.223	24.716	1.101
40	27.701	25.533	1.085
50	28.209	26.350	1.071
60	28.367	27.167	1.044
70	28.624	27.984	1.023
80	28.818	28.801	1.000

The data presented in Table 14 show that osmotic pressure increases with increase in temperature. The significance of the last two columns in this table will become apparent in the later discussion.

TABLE 15—OSMOTIC PRESSURES OF WEIGHT MOLAR SOLUTIONS OF SEVERAL ELECTROLYTES AND NON-ELECTROLYTES AS CALCULATED FROM FREEZING POINT DEPRESSIONS (DATA OF JONES, 1907)

Methyl alcohol	22.88 atmos.	KNO_3	32.87 atmos.
Ethyl alcohol	22.51	$Ca(NO_3)_2$	58.88
NaCl	42.74	K_2CO_3	52.61

The important fact illustrated by the data in Table 15 is that while the osmotic pressures of weight molar solutions of non-electrolytes approximate the same value, which is in the neighborhood of van't Hoff's theoretical value (see later) of 22.4 atmospheres, the values for weight molar solutions of electrolytes are much higher.

Using the results of Pfeffer's classical investigations of osmotic pressure, published in 1877, as a basis for his arguments, van't Hoff (1887) showed that a remarkable analogy exists between the osmotic pressures of dilute solutions and the pressures exerted by gases. Van't Hoff pointed out that the osmotic pressure of a dilute solution is equal to the pressure that the solute alone would exert were it present as a gas at the same temperature in the same volume as that occupied by the solution. A dilute solution was con-

sidered by van't Hoff to be one in which the volume of solute was small in proportion to the volume of solvent.[3] For example, if we could imagine the complete and instantaneous removal of all of the water in a sucrose solution without any change in the spatial relations of the solute molecules, the residual sucrose molecules would be in a gaseous state. According to van't Hoff the osmotic pressure of this solution would be equal to the gas pressure which would be exerted by such a hypothetical "sucrose gas." Furthermore, he showed that the osmotic pressures of dilute solutions obey laws analogous to those which describe the relations of gases to variations in temperature, volume and pressure. The analogies will now be briefly summarized:

(1) According to Boyle's Law the pressure exerted by a gas varies inversely as its volume, temperature remaining constant. In other words the pressure of a gas varies directly with its concentration, since the smaller the volume which contains a given number of gas molecules, the greater the concentration of the gas. A similar principle holds for osmotic pressures. A molar weight of an undissociated solute dissolved in 1000 g. of water, for example, results in a solution with twice the osmotic pressure possessed by a solution prepared by dissolving half the molar weight of the same solute in 1000 g. of water. The third column of Table 13 shows the calculated pressures which a "sucrose gas" would exert if dispersed in the same volume and at the same temperatures as the dissolved sucrose. The correspondence of these hypothetical "sucrose gas" pressures with the osmotic pressures of the sucrose solutions is evident from the last column of this table.

(2) According to the law of Gay-Lussac the pressure exerted by a gas varies directly with the absolute temperature, if the volume of the gas remains constant. From the data presented in the last two columns of Table 14 we see that the osmotic pressure of a sucrose solution shows approximately the same variation with temperature that the gas pressure of a corresponding hypothetical "sucrose gas" would show. The osmotic pressure of a solution is therefore proportional to the absolute temperature.

(3) According to Avogadro's Hypothesis equal volumes of all gases under identical conditions of temperature and pressure contain equal numbers of molecules and hence exert equal pressures. One mol of any gas at standard conditions (0° C.; 1 atmos. pressure) occupies 22.4 liters. If this gas be compressed to a volume of 1 liter, it will exert a pressure of 22.4 atmos. (Boyle's Law). Similarly one mol of any *undissociated* solute dissolved in a liter of water exerts an osmotic pressure of approximately 22.4 atmos. at 0° C.

[3] Actually the analogies between gas pressures and osmotic pressures as discussed by van't Hoff hold with a fair degree of approximation for solutions up to concentrations of at least one molar.

This is the same pressure that it would exert were it a gas confined in the same volume under standard conditions. It follows therefore that equal osmotic pressures of solutions of undissociated solutes indicate equal concentrations of solute molecules and that Avogadro's Hypothesis may be applied to osmotic pressures as well as to gas pressures. In other words the theoretical osmotic pressure of a weight molar solution of any undissociated solute is 22.4 atmos. at 0° C. The osmotic pressures of weight molar solutions of several undissociated compounds are given in the left-hand column of Table 15. It should be noted that these experimentally determined values agree only approximately with the theory. In more dilute solutions the values obtained are more nearly concordant with those required by this hypothesis.

Electrolytes, as first shown by DeVries in 1884, and as indicated by the data in Table 15, exhibit greater osmotic pressures than their molar concentrations would lead one to expect. This fact remained unexplained until the formulation of the ionization theory by Arrhenius in 1887. In fact DeVries' data were used by Arrhenius as strong evidence for his theory. Apparently each ion produced by the dissociation of an electrolyte is just as effective osmotically as an undissociated molecule. Hence all electrolytes produce higher osmotic pressures than the theoretical values for undissociated compounds; the exact magnitude of the pressures produced will depend upon their degree of dissociation. For example, a molar solution of NaCl is about 75 per cent dissociated. This means that 75 out of every 100 molecules of NaCl present have dissociated into ions. There will therefore be 1.75 times as many particles (ions and molecules) present as in a molar solution of a non-electrolyte. The osmotic pressure of a molar solution of NaCl would therefore be theoretically $22.4 \times 1.75 = 39.30$ atmos. Consultation of Table 15 shows that this value agrees approximately with the experimentally determined value for a molar NaCl solution.

The hydration of molecules or ions also influences the osmotic pressure of a solution. Water molecules which are bound to solute particles as water of hydration are no longer effective as a part of the solvent. In effect a solution containing hydrated solute particles is therefore more concentrated than its molarity would indicate. This accounts, at least in part, for the fact that the experimentally determined values of osmotic pressures are often in excess of the theoretical ones which would be expected according to the van't Hoff analogy with the gas laws. For example, a molar solution of sucrose should theoretically have an osmotic pressure of 22.4 atmos, at 0° C. Actually its measured pressure is found to be 24.83 atmos. (Table 14). This discrepancy is believed to be due to the hydration of the sucrose molecules. One sucrose molecule binds six molecules of water of hydration.

Hence one *mol* of sucrose will bind six *mols* of water. One thousand grams of water is equivalent to 55.5 mols of water (1000/18). Since the water of hydration is not free to act as a solvent, in effect the solution consists of 1 mol of sucrose dissolved in 49.5 mols of water. A simple calculation ($22.4 \times 55.5/49.5$) yields the value 25.12 atmos. as the theoretical osmotic pressure of a weight molar sucrose solution if hydration of the sucrose molecules is taken into account. This value is in close agreement with those which have been obtained experimentally.

Van't Hoff's demonstration of the analogy between osmotic pressure and gas pressure led many subsequent investigators to consider that osmotic pressure results from the impacts of the *solute* molecules against the interior wall of the membrane, an assumption which van't Hoff himself was careful to avoid. One reason for the persistence of this hypothesis of osmotic pressure has been the difficulty in understanding why—if the osmotic pressure is due to the solvent molecules—it should be approximately equal to the pressure which would be developed if the *solute* molecules were present in the same volume in the form of a gas.

The solvent pressure hypothesis holds that the osmotic pressure which develops in an osmometer or similar closed system is due to the excess of the inwardly directed diffusion pressure of the solvent over the outwardly directed diffusion pressure of the solvent. Haldane (1918) has shown that this excess inwardly directed diffusion pressure of the *solvent* is *mathematically* equal to the pressure which the *solute* molecules inside the osmometer would exert if present as a gas in the same volume as the solution. Thus it has become possible to reconcile the concept that osmotic pressure is due to the solvent molecules with van't Hoff's classical discoveries regarding the analogy between osmotic and gas pressures.

The Measurement of Osmotic Pressures.—The osmotic pressure of a solution, as such, can be measured only by the direct manometric method as employed by Pfeffer, Morse, and others. Precise results can be obtained by this method only at the cost of an elaborate technique and infinite precautions. Hence the actual number of measurements of osmotic pressures which have been made by this direct method are very limited. As is well known there is a direct proportionality between the osmotic pressure, lowering of the vapor pressure, elevation of the boiling point, and depression of the freezing point of solutions. Hence osmotic pressures of solutions can be calculated from the determined results of one of these three physical quantities. Most frequently determinations are made of the depression of the freezing point of a solution, and its osmotic pressure calculated from this. Since the theoretical freezing point depression of a weight-molar solution of an un-ionized

substance is 1.86° C., and the theoretical osmotic pressure of such a solution is 22.4 atmos., an equation relating freezing point depressions and osmotic pressures is easily derived. If we let Δ represent the freezing point depression of a solution, its osmotic pressure (O.P.) may be calculated as follows:

$$\text{O.P.} : 22.4 = \Delta : 1.86$$
$$1.86\ \text{O.P.} = 22.4\ \Delta$$
$$\text{O.P.} = \frac{22.4}{1.86}\Delta$$
$$\text{O.P.} = 12.04\ \Delta\ ^{4}$$

For example, a solution for which the determined freezing point depression is 0.930 would have an osmotic pressure of 11.10 atmos. (12.04×0.930). This equation appears to be approximately correct over a wide range of concentrations since deviations from the theoretical osmotic pressures of solutions are accompanied by almost strictly proportional deviations from their theoretical freezing point depression values. This method is often called the *cryoscopic* method of determining osmotic pressures. The osmotic pressures as determined by this method are as at the freezing point temperature of the solution.

Electro-osmosis.—Under certain conditions pure water will diffuse from one side of a membrane to the other under the influence of a difference of

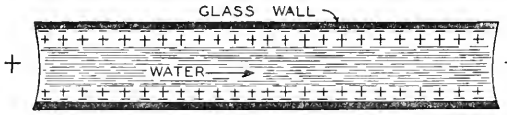

FIG. 21. Diagram to illustrate electro-osmosis.

electrical potential, a process which is called *electro-osmosis*. When water is confined in a capillary glass tube adsorption of OH^- (or HCO_3^-) ions imparts a negative charge to the walls of the tube . Adjoining them is a layer of H^+ ions equal in number to the adsorbed anions. In other words ions become distributed as an electrical double layer just as they do around a colloidal particle (Fig. 21).

If a difference of electrical potential is present between the two ends of the tube, water will travel towards the negative electrode. The anions are firmly adsorbed by the walls of the tube and cannot move. The hydrogen ions, however, are free to move and migrate toward the cathode carrying the water, of which they are a part, along with them. In other words the moving

[4] A slightly more accurate form of this equation has been derived by Lewis (1908). The tables of Harris and Gortner (1914, 1915), based on the Lewis equation, are very useful for converting freezing point depressions into osmotic pressures.

cations in the water glide past the stationary anions which adhere to the walls of the tube. Since many membranes are essentially porous in structure, conditions similar to those just described may exist in each capillary of the membrane, and electro-osmosis may occur through such membranes in essentially the same way that it occurs through tubes of small bore. Since differences of electrical potential are frequently present in living organisms it seems entirely probable that electro-osmotic flow of water occurs in living tissues.

Discussion Questions

In answering these questions use the theoretical value of 22.4 atmos. at 0° C. for the osmotic pressure of a weight molar solution of an undissociated solute, 1.86 as the freezing point depression of such a solution, and assume all membranes to be permeable to water only.

1. Distinguish between the osmotic pressure and the turgor pressure of a solution.
2. A closed sac-like membrane containing a solution with an osmotic pressure of 27 atmos. is immersed in a solution with an osmotic pressure of 16 atmos. In which direction will water move? Why? Assuming dilution of the internal solution to be negligible what will its turgor pressure be at equilibrium? What will it be if the osmotic pressure of the external solution is 22 atmos.? 30 atmos.?
3. The freezing point depression of a plant sap is found to be 3.72° C. What is its osmotic pressure at 25° C.?
4. The two arms of a U-tube are separated by a membrane permeable only to water. A solution with an osmotic pressure of 15 atmos. is placed in arm A, one with an osmotic pressure of 20 atmos. in arm B. Which way will water move? Explain.
5. If the solution in arm B (question 4) is subjected to a pressure of 10 atmos. which direction will water move? If a pressure of 5 atmos. is used? 3 atmos.? If the solution in both arms is subjected to a pressure of 8 atmos.? Explain.
6. If the solution in arm B (question 4) is warmed to 30° C. while that in A stays at 20° C., in which direction will water move? Why?
7. When in a flaccid (turgorless) condition a given plant cell has a volume of 10 (arbitrary units) and an osmotic pressure of 18 atmos. If in its fully turgid condition its volume is 15, what is its osmotic pressure? (Assume solution volume of solutes to be negligible.)
8. A 10 per cent solution of sucrose is enclosed in an osmometer with a vertical glass tube attached and the osmometer immersed in pure water. Disregarding dilution of the solution and assuming it has a specific gravity of 1, what is the approximate maximum height to which the liquid could rise in the tube?
9. Assuming 75 per cent ionization and no hydration what is the molar concentration of a KCl solution which exhibits an osmotic pressure of 22.4 atmos. at 0° C.?
10. An osmometer containing an 0.5 *volume molar* solution of sucrose is immersed in a beaker of water. After a time its volume is found to have doubled. What will then be the osmotic pressure of the solution inside of the osmometer?
11. How much sucrose in grams must be added to a liter of water in order to make a solution which has a freezing point of −4.28° C.?

12. An inverted U-tube has one arm filled with a solution with an osmotic pressure of 4 atmos. and the other arm with a solution the osmotic pressure of which is 7 atmos. Both open ends of the U-tube are closed with membranes permeable only to water and the portion of the tube above the solutions is filled with water. What will happen when: (a) both arms of the tube are dipped in water? (b) the 4 atmos. arm is dipped into a solution with an osmotic pressure of 1 atmos. and the 7 atmos. arm is dipped into a solution with an osmotic pressure of 4 atmos.? (c) a membrane permeable only to water is inserted into the curved part of the U-tube between the two arms and both arms dipped into water?

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CHAPTER IX

IMBIBITION

If a handful of the seeds of any species which do not possess seed coats impermeable to water be dropped into a vessel of water, within a few hours they will have swollen visibly. Many other materials will swell in a similar way when immersed in water. Among these are starch, cellulose, agar, gelatin, and kelp stipe. Some substances will swell similarly when immersed in other liquids. All of these phenomena are examples of the process usually called *imbibition*. The amount of water which may enter substances in imbibition is often very great in proportion to the dry weight of the substance which swells. A piece of dried kelp stipe, for example, can absorb as much as fifteen times its own weight of water.

Water may be imbibed as a vapor as well as in the liquid state. The swelling of doors and woodwork generally during damp weather is a familiar example of this phenomenon. Plant structures, if sufficiently low in water content, also imbibe water-vapor. The water content of "air-dry" seeds, for example, generally fluctuates with the vapor pressure of the atmosphere, rising with an increase in vapor pressure, and *vice versa*.

The Dynamics of Imbibition.—Imbibition is usually considered to be basically a diffusion process, but capillary phenomena are probably also involved. Imbibing substances are often permeated with minute submicroscopic capillaries, and it is impossible to determine how much of the liquid enters by diffusion and how much by capillary movement through invisible pores. Fundamentally, however, the cause of imbibition may be regarded as a difference in the diffusion pressure between the liquid in the external medium and the liquid in the "imbibant." As long as the latter is less than the former, movement of water into the imbibing substance will continue. An equilibrium will be reached, as in diffusion or osmotic phenomena, only when the diffusion pressure of the water in the two parts of the system has attained the same value.

A substance which imbibes water does not necessarily imbibe other liquids. Dry kelp stipe, for example, swells enormously when immersed in water, but does not swell when immersed in ether or other organic liquids. Con-

trariwise, a piece of rubber does not imbibe water, but does imbibe appreciable amounts of ether and other organic compounds when in contact with them in either the liquid or the vapor state. Obviously a difference in diffusion pressures between the liquid in an imbibant and in its surrounding medium is not the only requisite for the occurrence of imbibition. Certain specific attractive forces between the molecules of the imbibant and the imbibed liquid must also be present. In default of such specific attractions imbibition fails to occur, even if all other necessary conditions for the process are fulfilled. While perhaps not an invariable principle it appears to be generally true that only strongly polar substances (Chap. X) imbibe strongly polar liquids such as water, while non-polar substances such as rubber imbibe only non-polar or very weakly polar organic liquids.

Since in living organisms water is the only liquid imbibed, the further discussion will disregard other types of imbibitional phenomena. The physical relationship between the imbibed water and the imbibant is undoubtedly a complex one. The bulk of the imbibed water in any system is probably adsorbed on the molecules or micelles which constitute the structural units of the imbibing material. The possible relations of adsorbed water to the adsorbing particles in colloidal systems have already been considered (Chap. V). There is good reason for believing that similar physical relations exist between the imbibed water and unit particles in an imbibant. Such water may be pictured as either actually in solution in the adsorbing particles, or as adsorbed as a "shell" of from one to many molecular layers in thickness on their surface. It is possible that either or both of these conditions obtain in many systems containing imbibed water, but the second of these pictures appears to be more likely. It is also probable that a portion of the imbibed water enters and occupies minute submicroscopic capillaries which ramify between the component molecules or micelles of the imbibant. The result of imbibition is often the production of a system which in its essential properties may be regarded as a gel. The physical status of the water in a system into which it has been imbibed and in an elastic gel is undoubtedly very similar. In fact the imbibition of a dispersion medium by a dry substance is generally regarded as a method of gel formation.

Volume Changes in Imbibition.—The volume of the *imbibant* always increases during imbibition. The final volume of the entire system (liquid + imbibant) is always less, however, than the sum of the initial volume of the liquid and the imbibing substance. In other words a contraction in the volume of the system, liquid + imbibant, occurs during the process of imbibition. This volume shrinkage is not principally due to the occupancy of minute spaces within the substance of the imbibing material by the liquid, as might

be surmised, although in some systems a part of the contraction in volume possibly may be accounted for in this way. The explanation is undoubtedly to be ascribed to the fact that the adsorbed water molecules are definitely oriented in relation to the adsorbing surfaces, and hence occupy less space than when in the free state. This is equivalent to a compression of the adsorbed water so that its density is greater than that of free water.

Energy Relations of Imbibition.—The process of imbibition always results in the release of heat. Liberation of heat during imbibition may be easily detected by allowing dry starch or some other material with a high imbibitional capacity to imbibe water while contained in a calorimeter, and noting the change in temperature (Table 16).

TABLE 16—HEAT OF IMBIBITION OF DRY STARCH (DATA OF RODEWALD, 1897)

Per cent water imbibed	Heat evolved, g.-cal. per g. starch
0.23	28.11
2.39	22.60
6.27	15.17
11.65	8.43
15.68	5.21
19.52	2.91

The adsorption of water molecules which occurs when they are imbibed results in a loss of a large part of their kinetic energy, which reappears in the system as heat energy. This increase in the temperature of the system corresponds to an increase in the kinetic activity of all except the adsorbed molecules. The essential energy change in the process of imbibition is a loss of kinetic energy by the adsorbed molecules, and its transference to the other molecules of the system. Their increased kinetic activity is the cause of the observed rise in temperature. As shown in Table 16, the greatest evolution of heat accompanies the initial stages of imbibition. This is to be expected, as a larger proportion of the first molecules of water imbibed would be firmly adsorbed than of those which enter the imbibant later.

Factors Influencing Imbibition.—In discussions of the factors influencing imbibition it is often necessary to distinguish between the influence of a factor upon the *rate* of imbibition, and upon the total *amount* of water which will be held in the system when an equilibrium has been attained. The principal factors which influence the entrance of water into an imbibant are as follows:

1. *Temperature.*—The rate of imbibition increases with increase in temperature (Fig. 22). Equilibrium will therefore be attained more slowly at lower temperatures, but the amount of water held in an imbibant at equilibrium will be slightly greater at lower than at higher temperatures. This is in accord with the principles of the influence of temperature upon adsorption processes generally.

2. *Osmotic Effects.*—Water moves by imbibition into a substance only when its diffusion pressure is in excess of the diffusion pressure of the water

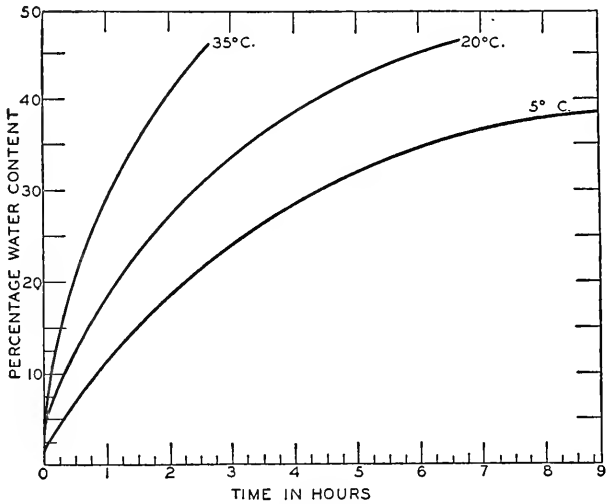

FIG. 22. Relation between temperature and imbibition of water by Xanthium seeds. Data of Shull (1920).

in the imbibant. The introduction of a solute into water invariably has the effect, as already discussed, of reducing the diffusion pressure of the water in that solution as compared with pure water. The osmotic pressure of a solution is a measure of its diffusion pressure deficit. Hence the magnitude of the osmotic pressure of the surrounding solution would be expected to influence both the rate of imbibition and the equilibrium water content of an imbibant.

One of the most comprehensive studies dealing with osmotic effects in relation to imbibition has been conducted by Shull (1916) who used the seeds of the cocklebur (*Xanthium pennsylvanicum*) as the imbibing material. Samples of the seeds of this species were allowed to come to equilibrium with solutions of sodium chloride and lithium chloride of different osmotic pres-

sures, after which the amount of water which had been imbibed was determined. The coats of these seeds are permeable to water, but virtually impermeable to these solutes. Were it not for this latter fact complicating effects due to the specific influences of the ions employed, as described in the next section, might invalidate the results of experiments of this type. The results of such a series of determinations are shown in Table 17.

TABLE 17—IMBIBITION OF WATER BY COCKLEBUR SEEDS IMMERSED IN SOLUTIONS OF DIFFERENT OSMOTIC PRESSURES (DATA OF SHULL, 1916)

Volume molar concentration of solutions	Osmotic pressure of solutions, atmos.	Water imbibed by seeds at equilibrium (48 hours). Per cent of air dry weight
H_2O	0.0	51.58
0.1 M NaCl	3.8	46.33
0.2	7.6	45.52
0.3	11.4	42.05
0.4	15.2	40.27
0.5	19.0	38.98
0.6	22.8	35.18
0.7	26.6	32.85
0.8	30.4	31.12
0.9	34.2	29.79
1.0	38.0	26.73
2.0	72.0	18.55
4.0	130.0	11.76
Sat. NaCl	375.0	6.35
Sat. LiCl	965.0	—0.29

The general principle shown by the results of these experiments is that with increase in the osmotic pressure of the solution in which the seeds are immersed, the amount of water held by imbibition per unit of dry weight at the equilibrium point decreases. Since at equilibrium the diffusion pressures of the water in the imbibing substance and in the surrounding liquid must be equal the basis for this osmotic effect upon imbibition is evident. If the diffusion pressure in the solution is relatively low (high osmotic pressure), the diffusion of less water into the imbibing substance is necessary to raise the diffusion pressure of the water in it to a value equal to the diffusion pressure of the water in the circumambient liquid, than if the diffusion pressure in the surrounding liquid is high. The relation between the osmotic pressure and the amount of water imbibed is not, however, a strictly proportional one. At the lower end of the range of concentrations employed, an increase of a few atmospheres in osmotic pressure causes a decrease in the

amount of water imbibed, in terms of the air-dry weight of the seeds, of nearly 15 per cent. Near the upper end of this range of concentrations an increase in osmotic pressure of several hundred atmospheres is required to produce an equivalent change in the volume of water imbibed. This is further evidence that the first increments of water passing into any imbibant are held by tremendously greater forces than those which are imbibed subsequently.

3. *Ionic Effects.*—The amount of water which will be imbibed from a given solution may also be influenced by the species of ions in that solution. If agar or kelp stipe, for example, is allowed to come to equilibrium with equimolar solutions of lithium, sodium, ammonium, and potassium chlorides swelling will not be equal in the different solutions. The greatest imbibition will occur in lithium chloride solution, next greatest in sodium chloride, next greatest in ammonium chloride, and least in the potassium chloride solution. In all of the solutions swelling will be less than in pure water (Fig. 23). At first sight it would seem that this might be ascribed to an osmotic effect. The potassium chloride solution might be assumed to have a higher osmotic pressure than the lithium chloride solution, due to differences in dissociation or hydration, and hence to exert more of a retarding effect upon imbibition than the other solutions. Actually the opposite condition holds, hence this influence of electrolytes upon imbibition cannot be explained in terms of an osmotic effect. Apparently, since all of these electrolytes give rise to the same anion the cause of this phenomenon must be looked for in terms of specific effects of the cations upon swelling.

Many other processes and physical properties are affected by this and similar ionic sequences. Such ionic series as these are known as *lyotropic series*, and such effects of ions as are found to be arranged in sequences of this sort are called *lyotropic effects*.

Two lyotropic series of cations, and one of anions, are generally recognized, as follows:

$$Li—Na—NH_4—K—Rb—Cs$$

$$Mg—Ca—Sr—Ba$$

$$CNS—I—NO_3—Br—Cl—SO_4$$

Sometimes the order of the effect of a lyotropic series is from left to right and sometimes the reverse depending upon the property affected. As arranged above the series of univalent cations represents (reading from left to right) the order of their increasing effectiveness in checking the imbibition of water by kelp stipe. The same is true of the series of bivalent cations. The anions in the third series do not differ very greatly in their effects upon

the swelling of kelp stipe, but are known to influence many other processes, the magnitude of their effects being in the order given.

These so-called lyotropic effects are apparently due largely to differences in the degree of hydration of various kinds of ions although other factors are probably also involved. Their lyotropic effect on the swelling of kelp stipe, agar, and other similar materials which bear a negative charge with respect

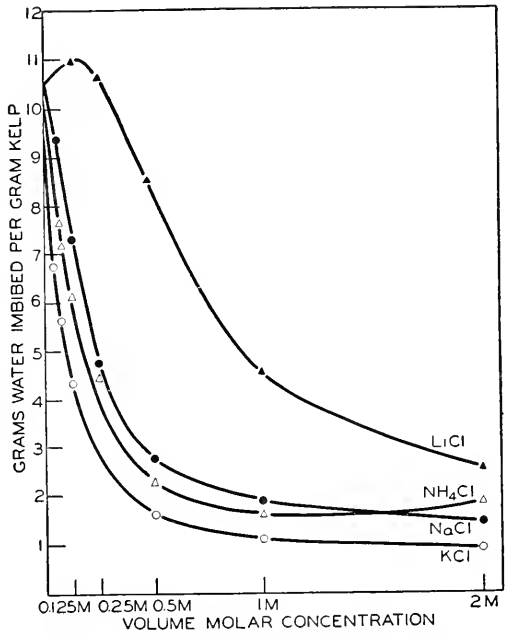

FIG. 23. Influence of cations on imbibition of water by kelp stipe. Data of Wagner (1936).

to water probably may be explained as follows: The negative charge on the kelp stipe is apparently due to adsorption of hydroxyl ions, leaving hydrogen ions in the outer zone of the electrical double layer. When immersed in a solution of an electrolyte not only is water imbibed, but an exchange of cations occurs, some of the cations in the electrolyte replacing hydrogen ions in the kelp stipe. The displaced hydrogen ions of the double layer pair off with the residual anions of the electrolyte. The water molecules associated with the cations which enter the double layer will contribute to the total *hydration* of the kelp. The different degrees of swelling caused by the different cations is probably due partly to the different numbers of water molecules

associated with individual cations of various species, and partly to the replacement of a larger proportion of hydrogen ions in the double layer by some species of cations than others.

In addition to this *lyotropic* effect upon swelling, ions often exert an *electrostatic* effect as well. Solutions of chlorides producing bi- and trivalent cations ($CaCl_2$, $AlCl_3$, etc.) result in a great reduction in the swelling of kelp stipe even when the concentration is so dilute that their osmotic effects are negligible. Exchange of the polyvalent cations for the H^+ ions in the double layer apparently results in a type of flocculating effect upon the negatively charged particles of the kelp. This brings the particles closer together and results in less swelling. Such effects are also exerted when polyvalent cations are associated with other univalent anions, but when paired with polyvalent anions the decreasing effect of salts producing polyvalent cations upon the swelling of negatively charged imbibants is less marked. There is a close similarity between this phenomenon and the effect of electrolytes in flocculating negatively charged sols.

4. *Effects of H-ion Concentration.*—Hydrogen ions in any appreciable concentration affect the swelling of a negatively charged imbibant adversely, while hydroxyl ions in any appreciable concentration cause greater swelling of such systems than occurs in pure water. Exactly the reverse effects are exerted by hydrogen and hydroxyl ions upon the swelling of positively charged imbibants. The swelling of amphoteric gel-forming substances such as gelatin and certain other proteins is influenced in an even more complex manner by the pH of the swelling medium as indicated in Fig. 24. Minimum swelling occurs at the isoelectric point, and with increase or decrease of pH from this point increased swelling occurs. At relatively low or relatively high pH values a decreasing effect upon swelling is again evident.

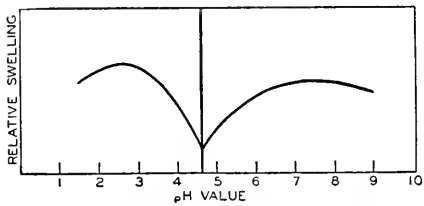

FIG. 24. Relation between pH and the swelling of gelatin (diagrammatic).

Imbibition Pressure.—Pressures, sometimes of an enormous magnitude, develop during the swelling of an imbibing substance. Such pressures only become evident if the imbibant is confined in some way during the process of imbibition. One common method of demonstrating the development of pressures during imbibition is as follows: A glass funnel, lined with filter paper, is partly filled with moist plaster of Paris paste. The surface of the plaster of Paris is then strewn with a number of pea seeds, after which more paste is added until the funnel is full. In a few minutes the moist matrix contain-

ing the seeds will have set. The resulting solid cone is removed from the funnel and its base immersed in a dish of water. Water moves upward through the porous gypsum cone by capillarity and permits a continued imbibition by the pea seeds. Within a few hours the pressure developed by the swelling pea seeds is sufficiently great to rupture the gypsum block.

The magnitude of the pressure developed during imbibition may be measured quantitatively in two different ways. It may be counterbalanced by a mechanical pressure, the magnitude of which becomes a measure of the imbibition pressure developed. Such a method was used by Reinke as long ago as 1879. He stacked disks of dried fronds of *Laminaria* (a sea weed) in a hollow metal cylinder, and inserted above the disks a metal piston bearing a platform at its upper end. Water was then brought into contact with the dry disks. By placing upon the platform weights of sufficient mass to just prevent swelling of the kelp, the magnitude of the imbibition pressure was determined. A second method of measuring this quantity relies upon counterbalancing the imbibition pressure of a substance by means of a solution of high osmotic pressure. The pressure developed by imbibition may be regarded as due to the diffusion pressure of the entering water. If the imbibing substance be immersed in a solution in which the diffusion pressure deficit (osmotic pressure) is sufficiently great to prevent any movement of water into that substance the osmotic pressure of that solution is a measure of the imbibition pressure which would be developed in that imbibant. In Table 17 the data show that a solution with an osmotic pressure of nearly a thousand atmospheres was required to prevent any imbibition of water by cocklebur seeds. As determined by either of the two methods just described the imbibition pressure of an imbibant is analogous to the osmotic pressure of a solution, *i.e.* it is a measure of the maximum imbibition pressure which the imbibant can develop.

Discussion Questions

1. How could you decide whether the swelling of dry prunes in water is the result of osmosis or of imbibition?
2. When equal volumes of starch and water are used why is less heat liberated when air dry corn starch is mixed with water than when corn starch which has been dried in a desiccator over sulfuric acid is used?
3. Would you expect gelatin and plant cell walls to show similar swelling behavior when immersed in a series of solutions of different pH?
4. In a system composed of kelp stipe or wheat seeds plus water would you expect shrinkage in volume of the system to be greatest at 10° C. or at 40° C.? Explain.
5. How would you expect cations to affect the swelling of an imbibant in which the micelles are positively charged? anions?
6. If pieces of kelp stipe are fastened to the base of a hydrometer which is then immersed in a vessel of water the hydrometer will sink as the kelp swells. Explain.

Suggested for Collateral Reading

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CHAPTER X

PERMEABILITY

According to the principles of diffusion, the molecules and ions of all substances in solution, and even colloidal micelles, tend to attain an equal distribution in terms of concentration through all parts of a system. The same is true of gases, and the osmotic movement of water, as we have seen earlier, is a response to the same general principle. In living organisms, however, such an equality of distribution of the particles of a solute among the various cells, or between cells and a liquid environment, is seldom attained. There is a marked heterogeneity in the distribution of solutes among the different cells in tissues, in entire organisms, and even among the different parts of the same cell. The diffusion of substances in solution into or out of plant cells is limited and modified in a number of ways. The diversity of the distribution of solutes within the plant body is a result of conditions which modify or check their free diffusion.

The *permeability* of the cell, or some part thereof, is one of the most important factors influencing the rate with which molecules and ions diffuse into or out of plant cells. Plant cells and parts of plant cells such as nuclei and plastids are bounded by membranes which are notable for their differences in permeability to different substances. The permeability of a membrane to a given substance is measured by the rate at which that substance will pass through that membrane under a given "driving force." In the simplest cases, this driving force depends upon the steepness of the concentration or diffusion pressure gradient of the diffusing substance across the membrane.

The concept of permeability is inseparable from the idea of a membrane. Permeability, it should be clearly understood, is a property of the *membrane* and not of the substance which diffuses through it. Common examples of non-living membranes, some of which have already been described in previous chapters, are thin sheets or layers of rubber, collodion, parchment paper, cellophane, gelatin, and copper ferrocyanide. Liquids may also serve as membranes under certain conditions, as an example described later in this chapter shows.

Most biologically important membranes are of the type characterized as

differentially permeable. Different substances diffuse through such membranes at different rates. They may be, and often are, impermeable or virtually so to some substances, while others pass through them readily. Such membranes are sometimes termed *selectively permeable* or *semi-permeable,* but these two terms are not as truly descriptive of their properties as the designation "differentially permeable."

The Membranes of Plant Cells.—With very few exceptions the protoplasm of plant cells is enclosed by a definite, more or less rigid cell wall. This cell wall fulfills all the criteria for being considered a membrane. Through it must pass all substances moving either into or out of a cell. The cell wall membrane is sometimes called the "non-living membrane" of a cell, but the assumption that all cell walls are entirely non-living is scarcely a valid one. The plasmodesms, at least, must be considered in any problem involving the permeability of the cell walls of living plants.

Lining the cell wall of all mature plant cells is the layer of cytoplasm. The cytoplasm, or parts thereof, comprises the second membrane through which substances entering or leaving the vacuole of a cell must penetrate. This layer is sometimes called the "living membrane" of the cell. Both theoretical considerations and experimentally observed facts support the view that the two limiting layers of the cytoplasm possess different physicochemical properties from the interior of the cytoplasm and actually may be regarded as distinct membranes. The cytoplasmic membrane adjacent to the cell wall is called the *plasmalemma;* that enclosing the vacuole the *tonoplast* or *vacuolar membrane.* Since the boundaries between the cytoplasm and the cell wall and the cytoplasm and the vacuole constitute interfaces, protoplasmic ingredients which lower interfacial tension probably become more concentrated in these layers than in the interior of the cytoplasm. For this reason alone the postulation that the cytoplasmic membranes possess different properties from the interior cytoplasm seems justified.

Results from several types of experimental investigations support this hypothesis. The immiscibility of protoplasm with water, already discussed, appears to be at least partly due to the fact that protoplasm is coated with a layer of material which is insoluble in water. By means of micropipettes certain non-toxic dyes can be injected into the body of the cytoplasm through which they will spread rapidly. They do not, however, pass through either the plasmalemma or the tonoplast. Neither will they pass into the interior of the cytoplasm from a solution bathing the cell, nor from the vacuole if the latter is injected with the dye (Plowe, 1931). This is direct evidence that the constitution of the plasmalemma and the tonoplast is different from that of the interior cytoplasm. The presence of cytoplasmic membranes

can also be demonstrated by the manipulation of living protoplasm with fine glass needles (Chambers and Höfler, 1931). It has been found possible to withdraw a plasmolyzed protoplast (Chap. XI) from its cell wall and to strip off the protoplasm from the vacuole leaving the latter as a free-floating sac full of cell sap enclosed by a delicate membrane—the tonoplast (Fig. 25). Most evidence indicates that the tonoplast and probably also the plasmalemma are thin liquid films which are immiscible with water.

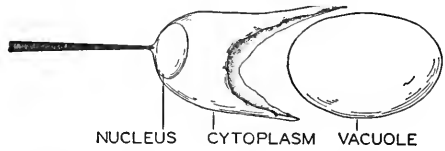

FIG. 25. Removal of the outer cytoplasm from the tonoplast and vacuole of a plasmolyzed cell. Redrawn from Seifriz (1928).

A complete picture of the permeability of a cell to different compounds can be drawn only in terms of the permeability of both the cell wall and the cytoplasmic membranes. In entering a cell a substance must first pass through the various layers of the cell wall, thence in turn through the plasmalemma, the interior cytoplasm, and the tonoplast before reaching the vacuole. Although some investigators prefer to refer all permeability phenomena of plant cells to the cytoplasm as a whole, the evidence that the plasmalemma and the tonoplast exist as distinct membranes with the property of differential permeability must be regarded as very substantial.

It is also customary to speak of certain plant structures composed of one or more layers of cells as membranes. Examples are the seed coats of many seeds and fruits (especially grains), epidermal layers such as those peeled from the scales of an onion bulb, the "skin" of potatoes, etc. Such membranes may be termed for convenience *multicellular membranes*. They may be either living, as for example onion epidermis, or non-living, as for example many seed coats.

The Permeability of the Cytoplasmic Membranes.—The cytoplasmic membranes are differentially permeable membranes *par excellence*. The term "cytoplasmic membranes" will be employed in a loose sense to refer to the entire cytoplasmic system of membranes: plasmalemma plus interior cytoplasm plus tonoplast. Actually many substances entering plant cells are intercepted and utilized in one way or another in the cytoplasm, and never diffuse through the tonoplast. Similarly compounds synthesized in the cytoplasm may pass out of the cell without crossing the tonoplast. Hence the plasmalemma is often regarded as the most important unit in the cytoplasmic system of membranes.

A pertinent concept as a background for a discussion of the permeability of the cytoplasmic membranes is the distinction between "polar" and "non-

polar" compounds and groups (Lewis, 1916). Water, electrolytes, carbohydrates, and amino acids are typical strongly polar compounds. This "polarity" is fundamentally an electrical phenomenon and is due to the grouping of the atoms in a molecule, as well as to the actual structure of its constituent atoms in terms of the spatial arrangement of protons and electrons. A polar molecule can be likened to a tiny magnet since it possesses certain regions which are electro-positive, while other regions are electro-negative (Fig. 26, *A*). This property of polarity is more strongly marked in some species of molecules than in others. Electrolytes possess the most strongly polarized of all molecules; the production of ions by such compounds may be considered a manifestation of a marked polarity within the molecules (Fig. 26, *B*). Polar compounds are, in general, more soluble in polar solvents, of which water is the most familiar example, than in non-polar solvents.

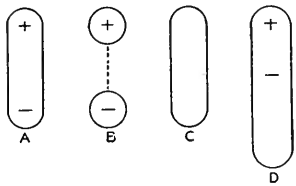

FIG. 26. Diagrams illustrating: (*A*) polar molecule, (*B*) ionized polar molecule, (*C*) non-polar molecule, (*D*) molecule which is polar at one end; non-polar at the other.

When the structural framework of molecules and their constituent atoms is such that no markedly contrasting electro-positive and electro-negative regions are present they are classed as non-polar (Fig. 26, *C*). Such molecules are electrically inert. The most typical non-polar compounds are the hydrocarbons of both the chain and ring types, such as methane (CH_4) and benzene (C_6H_6). Non-polar compounds are much more soluble in non-polar solvents such as benzene, chloroform, and ether than in polar solvents such as water.

The concept of polarity applies not only to molecules as a whole, but to certain atomic groupings within molecules. Such groups as CH_3—, C_2H_5—, etc., are non-polar, while —COOH, —OH, —NH_2, —CHO, —CN are the more familiar of the polar groupings. The molecules of many types of compounds, as for example the organic acids, contain both polar and non-polar groups. One end of an acetic acid (CH_3COOH) molecule, for example, is decidedly polar, while the other is essentially non-polar (Fig. 26, *D*). In general, the greater the number of non-polar groups which a molecule contains in proportion to the number of polar groups present the more predominant will be the non-polar properties of the molecule and *vice versa*.

One of the few important generalizations which may be made regarding the permeability of the cytoplasmic membranes is that they are usually much more permeable to predominantly non-polar than to predominantly polar molecules.

Electrolytes are all strongly polar compounds, hence it is not surprising to find that the cytoplasmic membranes of many plant cells are not readily permeable to them. However, as described later in this chapter, and still more fully in Chap. XXIV, electrolytes often enter certain kinds of plant cells readily. The actual mechanics of their penetration through the cytoplasmic membranes is a more complex process than simple diffusion. Some authorities contend that electrolytes penetrate as ions; others that they penetrate only as molecules. For the purpose of this discussion the first of these two viewpoints will be adopted.

Univalent cations such as Na^+ and K^+ usually penetrate through the cytoplasmic membranes more readily than bi- and trivalent cations such as Ca^{++} and Al^{+++}. The same general relationship also seems to hold for anions.

Among non-electrolytes in general the less polar the molecules, the more readily they can penetrate through the cytoplasmic membranes. Hydrocarbons such as benzene, being the least polar of all compounds, penetrate into cells very rapidly, although in only very small quantities, because of their low solubility in water.

The cytoplasmic membranes are usually quite permeable to the gases carbon dioxide and oxygen when in the dissolved state. This is in accord with the general principle since the molecules of these gases are essentially nonpolar. When carbon dioxide dissolves in water a large proportion (more than 50 per cent) of the molecules present react with the water, forming carbonic acid (H_2CO_3). This is a complicating factor which must be considered in any evaluation of processes involving the diffusion of dissolved carbon dioxide.

Most organic compounds containing only one polar group such as —OH, —COOH, or —NH_2 diffuse across the cytoplasmic membranes less rapidly than those containing no polar groups, but more rapidly than compounds containing two polar groups. Further increase in the number of polar groups present in a molecule still further decreases the penetrability of the molecule. Glycerol ($C_3H_5(OH)_3$), for example, an alcohol containing three polar —OH groups, penetrates relatively slowly, while monohydric alcohols such as methyl (CH_3OH) and ethyl (C_2H_5OH) penetrate through the cytoplasmic membranes very rapidly.

Most studies indicate that the cytoplasmic membranes of most kinds of cells are usually relatively impermeable to sugars. Impermeability to sucrose is especially marked (Bonte, 1934, and others). Since molecules of sugars contain a large number of polar groups (see the structural formulas for hexoses and sucrose in Chap. XXII), this is in accord with the general conception of the relation of the polarity of molecules to the permeability of the

cytoplasmic membranes to them. However, as in the case of electrolytes, there are some kinds of cells which sugars enter relatively rapidly.

Amino acids (Chap. XXVI), another extremely important group of compounds physiologically, also penetrate through the cytoplasmic membranes of most cells relatively slowly. Since each amino acid molecule contains one or more —NH_2 groups and one or more —COOH groups the behavior of these compounds is in accord with the general principle.

Water molecules, although decidedly polar, are an exception to the general rule, usually penetrating through the cytoplasmic membranes with relative ease. This is probably possible only because of the relatively small size of the water molecule. That cytoplasmic membranes are often, at least, quite permeable to water is shown by the frequent rapid loss or gain of water by plant cells.

Another important difference between permeability to polar compounds and to non-polar compounds is that ease of penetration of the former shows a close correlation with particle size, while this is not true of the latter. As among different ions of the same electrostatic charge, for example, the smaller ions (due account being taken of hydration) seem to penetrate more rapidly.

No such correlation is evident between particle size and permeability among molecules of a predominantly non-polar structure. In fact, exactly the reverse relation sometimes holds. For example, in the saturated series of fatty acids the larger the number of non-polar groups present, *i.e.* the greater the size of the molecule, at least up to a certain limit, the greater the permeability of the cytoplasmic membranes to the acid. According to Loeb (1909) the order of penetrability of the saturated fatty acids into sea urchin eggs is as follows, the most rapidly penetrating acid being given first: nonylic ($CH_3 \cdot (CH_2)_7 \cdot COOH$), caprylic ($CH_3 \cdot (CH_2)_6 \cdot COOH$), caproic ($CH_3 \cdot (CH_2)_4 \cdot COOH$), butyric ($CH_3 \cdot (CH_2)_2 \cdot COOH$), proprionic ($CH_3 \cdot CH_2 \cdot COOH$), acetic ($CH_3 \cdot COOH$) and formic ($H \cdot COOH$).

Permeability of the cytoplasmic membranes to polar compounds differs from permeability to non-polar compounds in one other respect. Permeability to the former is markedly influenced by external factors and by internal changes in the membrane. The same membrane apparently may vary all the way from a condition of marked permeability to certain polar solutes to a condition of almost complete impermeability to the same solutes. Permeability to non-polar solutes, on the other hand, is not only relatively high, but much more nearly constant, and much less influenced by environmental factors.

Factors Affecting the Permeability of the Cytoplasmic Membranes.— A number of factors of external origin may influence the permeability of the cytoplasmic membranes. Only the more important of these will be considered.

As the preceding discussion has indicated environmental factors influence the permeability of the cytoplasmic membranes to polar compounds more markedly than to non-polar compounds.

1. *Temperature.*—The apparent permeability of membranes to many substances increases with temperature. In part this effect may be due to increased kinetic activity of the molecules which pass through the membrane rather than to any effect of temperature on the membrane itself. A given substance will penetrate through a membrane more and more rapidly as the temperature is raised, until the membrane itself is destroyed, or profoundly modified by the temperature attained. In living membranes the upper limit of temperature which results in the destruction of the membrane is the thermal death point of the protoplasm which in most active cells usually lies between 50 and 60° C. At this point a sudden, irreversible increase in permeability occurs. This is due to death of the protoplasm and consequent destruction of the property of differential permeability in the cytoplasmic membranes. This phenomenon may be observed readily by heating a small piece of red beet tissue in a test tube of water. As soon as the thermal death point of the protoplasm is exceeded, the red pigment dissolved in the cell sap (an anthocyanin), to which the living cytoplasmic membranes are impermeable, rapidly diffuses out into the water.

Temperatures low enough to induce ice formation in plant tissues also result in an increase in the permeability of the cells of many (but by no means all) tissues, due to the resultant destruction of the protoplasm (Chap. XXXIII). This effect can be conveniently illustrated by freezing a small block of red beet tissue and immersing it in water as soon as it has thawed. The almost immediate outward diffusion of anthocyanins which ensues is evidence of the destruction of the cytoplasmic membranes.

2. *Electrolytes.*—According to Osterhout (1922) investigations made on the tissue of *Laminaria* (a kelp) by the conductivity method [1] indicate that the cations Li^+, Na^+, K^+, NH_4^+, Cs^+, and Rb^+ all increase permeability. This seems to be true regardless of the anion with which the cations are paired. The cations are arranged from left to right in their probable order of effectiveness in increasing permeability. This, it will be noted, is approximately the order of the lyotropic series.

The initial effect of bivalent and trivalent cations such as Ca^{++}, Ba^{++}, Sr^{++}, Mg^{++}, Fe^{++}, La^{+++}, Fe^{+++} and Al^{+++} as found by this method

[1] In this method permeability is determined by measuring the electrical conductivity of cylinders or stacks of disks of plant tissue. The greater the electrical conductivity, the greater the permeability. Since the conductivity of the tissue is due to the movement of ions through it, this method measures permeability to electrolytes only.

is a decrease in permeability. This primary effect is usually followed in time by a secondary increasing effect upon permeability. These results were found when these cations were associated with univalent anions. When the anions are of higher valence the initial decreasing effect upon permeability may be diminished or entirely offset.

Similar effects of univalent and bivalent cations on the permeability of the cytoplasm of the epidermal cells of onion bulb scales to water are recorded by de Haan (1935).

Raber (1920), using the same method and type of material as Osterhout, studied the effects upon permeability of a number of salts composed of the same cation (Na^+) but different anions. All of these salts were found to result in an increase in permeability. When supplied in equimolar concentrations the order of the increasing effect of the anions upon permeability was: citrate $>$ PO_4 $>$ tartrate $>$ SO_4; and acetate $>$ Cl $>$ NO_3 $>$ Br $>$ I $>$ CNS. The influence of the four polyvalent anions in increasing permeability was much more marked than the univalent anions. Both of these series follow closely the lyotropic order as usually given for anions.

The effect of any electrolyte upon permeability can be analyzed only in terms of the anions and cations into which it dissociates. The results of conductivity experiments seem to indicate that the following general principles hold (Raber, 1923): If the valency of the anion exceeds that of the cation, the salt results in an increase in permeability. If the converse is true the initial effect of the salt is a decrease in permeability; this is followed by a secondary increasing effect. When cation and anion are of the same valence, the effect will depend upon the relative size of the ions and other factors. When both ions are univalent, however, the usual effect is an increase in permeability.

The interpretation of these effects of electrolytes upon permeability is not entirely clear, but if we assume the cytoplasmic membrane to be composed of negatively charged micelles, an explanation similar to that given in the preceding chapter for the influence of ions upon imbibition would seem to fit the facts closely.

Not all studies of the effects of ions upon permeability have yielded results in accord with those just discussed. For example Brooks (1927) found that sodium, potassium, magnesium, and calcium chlorides in concentrations of 0.0125 to 0.05 molar all check the penetration of the dye "dahlia" into living cells of *Nitella*. Similarly Iljin (1928) found that dilute solutions of the above salts as well as several others all result in a decrease in the permeability of the cytoplasmic membranes of the cells of various species to sugars. The effects of electrolytes upon permeability of the cytoplasmic membranes prob-

ably varies not only with their concentration but also with different types of cells, and with the same cell under different conditions.

The effects of a mixture of two entirely different salts upon permeability should also be considered. This is illustrated in an experiment described by Bayliss (1924). A slice of freshly cut red beet was immersed in a solution of 0.31 M sodium chloride. It was observed that the red pigment gradually diffused out into the solution. Since when similar slices were immersed in water no such outward diffusion of red pigment occurred this showed the effect of sodium chloride in increasing permeability. If, however, enough calcium chloride was added to a portion of the sodium chloride solution before the slice of beet was placed in it to bring the concentration of the former salt to 0.17 M no such outward diffusion of red pigment could be detected. Evidently the presence of Ca^{++} ions in some way offset the usual effect of Na^{+} ions upon permeability. Such an effect of one ion upon another is known as *antagonism* (see also Chap. XXV).

3. *Non-electrolytes.*—Certain non-electrolytes are known to have effects upon permeability. Among these are the anaesthetics. Exposure of the cytoplasmic membranes to extremely low concentrations of ether or chloroform has no apparent effect upon their permeability; exposure to slightly higher concentrations decreases their permeability. With exposure to still higher concentrations this decreasing effect on permeability is only temporary, and is followed by a rapid increase in permeability which may continue until the value characteristic for the death point of that tissue is reached. This secondary effect of anaesthetics is due to the toxic effect of high concentrations upon the protoplasm resulting in its death after a relatively short period of time. The initial decreasing effect of anaesthetics upon permeability is reversible, but the secondary increasing effect is usually non-reversible.

4. *Hydration.*—Changes in the osmotic or imbibitional relations of the water in other parts of the cell may induce changes in the hydration of the cytoplasmic and cell wall membranes. It is inferred that variations in the permeability of the cytoplasmic membranes occur with shifts in their water content. Such changes in permeability are often presumed to be related to changes induced in the viscosity of the cytoplasm due to variations in its degree of hydration. Extreme desiccation of most cells results in a coagulation of the protoplasm, and a consequent complete loss of the property of differential permeability by the cytoplasmic membranes. In some cells, however, particularly those of drought resistant plants, the cytoplasm may regain its normal condition of differential permeability when water again becomes available after a period of practically complete desiccation.

5. *Light.*—As shown by the work of Lepeschkin (1930) and others, ex-

posure to light results in an increase in the permeability of the cytoplasmic membranes to many and perhaps to all solutes. It is uncertain, however, just how much of this effect can be ascribed to a direct photo-chemical influence and how much is due to secondary effects such as heating.

6. *"Stimulation."*—This ignorance-concealing term is applied to a number of phenomena which result in an increase in permeability, the exact nature of which are so incompletely understood that they cannot be described in any more fundamental terms. For example, it has been found that the permeability of the egg cells of certain species of invertebrate animals increases when fertilization occurs. Likewise injury or wounding of cells or tissues, whether due to mechanical or other causes, also usually results in an increase in permeability.

The Permeability of the Cell Wall Membranes.—The permeability of the cell wall membranes is greatly influenced by their chemical composition and physical organization. Cell walls composed of cellulose and pectic compounds, such as those in most parenchymatous tissues, are usually quite permeable to water, and, if wet, to many dissolved substances. Water and solutes probably pass through such walls largely if not entirely through the hydrophilic intermicellar material. The plasmodesms also undoubtedly facilitate the movement of water and solutes through the walls of many living plant cells.

That the differentially permeable membrane of such plant cells lies in the cytoplasm is visually shown when the protoplasm of any cell which contains anthocyanins dissolved in the cell sap is destroyed by heating, freezing, or toxic agents. As long as such a cell is alive and uninjured the red pigment does not diffuse out of the vacuole, even if the cell is immersed in water. Immediately upon destruction of the protoplasm, however, outward diffusion of anthocyanins and other solutes occurs through the intact cell wall, indicating that the latter does not appreciably impede their egress from the cells.

Lignified cell walls are also quite freely permeable to water and, when wet, to solutes as well. Lignification apparently results in little if any decrease in the permeability of the intermicellar material.

Cell walls in which cutin or suberin are present in appreciable quantities, on the other hand, are relatively impermeable both to water and solutes.

While it appears to be generally true that cell wall membranes which are readily permeable to water are also readily permeable to solutes dissolved in water, a number of differentially permeable cell wall membranes have been discovered and it is probable that differential permeability is more often a property of the cell walls than is generally realized. The coats of a number of different kinds of seeds and fruits have been found to act as differentially

permeable membranes. These coats may be several cell layers in thickness, but the cells of which they are composed are often dead. Even in those in which the cells are living destruction of the protoplasm by one treatment or another does not destroy the property of differential permeability. Clearly therefore, this property is inherent in the cell walls. Many such membranes are relatively permeable to water but show different degrees of permeability to solutes, ranging all the way from practical impermeability to some to a marked permeability to others. Among the species whose seed or fruit coats have been found to show this property are many of the grains, peach, apple, bean, horse-chestnut, sugar-beet, sunflower and cocklebur. For example, Shull (1913) found that the seed coat of the cocklebur was impermeable to ether, acetone, chloroform, ethyl alcohol, glycerol, sugars, NaCl, $CuSO_4$, HCl, and tartaric acid among others, but was permeable to a greater or lesser degree to KCl, KOH, NaOH, H_2SO_4, HNO_3, $AgNO_3$, $NaNO_3$, acetic, citric, and lactic acids, as well as some others.

Relation between Permeability and the Rate of Absorption.—There is not necessarily any correlation between the permeability of the cell membranes to a given compound and the rate at which that substance will enter the cell from other cells or a liquid medium. The permeability of the cell membranes does not control the rate at which compounds pass either into or out of a cell but is merely one factor which influences their rate of movement through the cell wall and the cytoplasm. For example, the membranes of a cell may be readily permeable to a given substance which nevertheless may enter very slowly because the vacuolar concentration of that substance is already relatively high. Another substance, to which the membranes are relatively impermeable, may move into the cell continuously because it is being utilized in some metabolic process within the cell.

Recent work on the absorption of ions by plant cells (Chap. XXIV) indicates that the results of many studies upon permeability to electrolytes are somewhat misleading. In actively metabolizing cells ions often accumulate until their vacuolar concentration is much greater than in the outside medium. The penetration of electrolytes into such cells often occurs at a relatively rapid rate, in spite of much evidence which indicates that the cytoplasmic membranes are not very permeable to them. Similarly there is evidence that sugars sometimes move into at least certain kinds of cells at a relatively rapid rate. A probable explanation of this apparent discrepancy is that most studies of permeability have been made upon cells which do not possess the capacity of absorbing electrolytes or other polar compounds at a rapid rate. This capacity seems to be restricted largely, if not entirely, to meristematic or otherwise rapidly metabolizing plant cells.

Theories of Membrane Permeability.—A number of hypotheses have been proposed as an explanation of the mechanism of differential permeability in both inorganic and living membranes. The discussion will be limited largely to the bearing of these theories upon the permeability of the cytoplasmic membranes. The two theories which have been most favorably regarded in all discussions of this topic are the sieve theory and the solubility theory. A number of variants of each of these theories has been proposed.

1. *Sieve Theory.*—Probably the simplest picture which can be conceived of the operation of a membrane is that it works as a molecular sieve. The pores in the membrane may be imagined to be of such dimensions that micelles, molecules or ions below a certain size can diffuse through them while those exceeding this size limit cannot pass through. Certain types of inorganic membranes such as collodion, parchment paper, and copper ferrocyanide undoubtedly owe their property of differential permeability largely to such a sieve-like action.

Although a sieve theory of the permeability of the cytoplasmic membranes is strongly advocated by some authorities, notably Bayliss (1924), there are some very grave objections to the acceptance of any such hypothesis as a complete explanation of this phenomenon. It is difficult to conceive of a sieve-like structure of the cytoplasmic membranes, except insofar as this is imparted by the normal intermolecular distances which are of course, very minute. A more serious difficulty, however, is found in the fact that the permeability of the cytoplasmic membranes to certain classes of compounds actually *increases* with increase in the size of the molecule, at least up to a certain point. This is true in certain homologous series of organic compounds already cited.

2. *Solubility Theories.*—A second generally advocated theory is that membranes are permeable to substances which will dissolve in them and impermeable to others. The operation of an inorganic membrane of this type can be readily demonstrated by a simple experiment. A test tube is about half filled with chloroform on top of which is introduced a very thin layer of water, and then nearly filled with ether. The water may be colored faintly with a water-soluble dye such as methylene blue in order to aid in distinguishing it from the other liquids. After stoppering, the tube is clamped in an upright position, and the position of the water meniscus is marked accurately. A second tube is prepared in exactly the same way, except that xylene is substituted for ether as the upper layer of liquid. If examined after a period of several days, it will be found that in the first test tube the level of the water meniscus has risen, while in the second it has fallen, although the distance through which the meniscus will move will not be as great in the second tube as in the first. In the first tube ether is diffusing through the water membrane more rapidly

than chloroform, hence the volume of liquid below the membrane is constantly increasing, and the layer of water rises. In the second test tube the chloroform diffuses through the water membrane more rapidly than the xylene, hence the position of the layer of water is lowered. This behavior is in accordance with the known solubilities of these three compounds in water; ether is the most soluble, chloroform next, and xylene the least. Evidently in this experiment permeability of the water membrane to these three compounds is directly correlated with their solubility in water.

The most familiar form of the solubility theory as applied to cytoplasmic membranes is the "lipoid solubility" theory first proposed by Overton (1904). In brief this theory holds that substances soluble in lipoids pass readily through the cytoplasmic membranes while others do not. The term "lipoid" is used in a loose way to refer to fats and fat-like compounds. In recent usage the term "lipid" has been rather generally substituted for "lipoid" (Chap. XXIII). Several lines of evidence appear to favor this theory. Among these are: (1) the non-miscibility of protoplasm with water, which probably indicates the presence of a film of fatty material on the outer surface of the protoplasm, (2) the fact that most lipoids lower surface tension, and hence theoretically would accumulate at interfaces, and (3) the high resistance of protoplasm to the passage of an electrical current, which indicates that it contains layers through which ions do not diffuse readily. A fourth line of evidence for this theory comes from Overton's own experiments. He found an almost perfect correlation between the lipoid solubility of hundreds of organic compounds and the permeability of the cytoplasmic membranes to them. On the other hand, sugars, salts, and other lipoid-insoluble substances showed practically no penetration into the cells at all. We now know that the terms lipoid-soluble and lipoid-insoluble correspond very closely to the modern terms of non-polar and polar, respectively.

While Overton's experiments seemed to show that the cytoplasmic membranes are relatively impermeable to polar compounds, later work has not all supported this generalization. It is true that polar compounds often penetrate with much greater difficulty than non-polar compounds and that the permeability of a given membrane to them may fluctuate, but the membranes are, at times at least, permeable to them. Moreover on *a priori* grounds it is evident that the membranes must be permeable to sugars, salts, etc. at least part of the time, since in the majority of cells most of these compounds present must have entered the cell at some time or another during its life history.

Some investigators hold that the lipoid solubility theory is essentially a correct representation of the mechanism of cytoplasmic permeability. Polar compounds would not be completely insoluble even in a lipoidal layer, and

hence could penetrate through the membrane at relatively slow rates. Osterhout (1936) considers that electrolytes must diffuse through this layer principally in the form of molecules as their dissociation when dissolved in a fat-like liquid would be very slight.

Other authorities are not convinced that this theory presents a complete picture of the basis for differential permeability in the cytoplasm. Observed rates of penetration of some polar compounds seem too great to be accounted for satisfactorily by the lipoid-solubility hypothesis. Modifications of this theory have therefore been suggested. For instance Nathansohn (1904) has proposed a theory which postulates a membrane composed of a mosaic of proteins and lipoids. He assumed that polar (lipoid-insoluble) compounds could diffuse through the more or less hydrated protein portion of the membrane. Permeability to the latter class of compounds might vary with changes in the hydration of the proteins. Although this view is almost purely speculative, some properties of the membrane, such as its elasticity, suggest the presence of proteins as one of its constituents.

3. *Adsorption and Chemical Reaction Theories.*—It has been suggested that the permeability to non-polar compounds is correlated more closely with the adsorptive capacity of the particles of the membrane for them than to their solubility in membrane constituents. In brief this *adsorption theory* would hold that the more strongly molecules are adsorbed, the more readily they will pass through the membrane. The possible mechanics of the transport of adsorbed molecules across a membrane is described later.

Chemical reactions may be involved in the transport of certain types of solutes across the cytoplasmic membranes. A penetrating molecule or ion, according to such a *chemical reaction theory*, is supposed to combine with some substance at the outer boundary of the membrane, thus forming an intermediate compound, in the form of which it is presumed to move across the membrane. Once the other boundary of the membrane has been reached a second chemical reaction is supposed to occur resulting in release of the ion or molecule on the interior of the membrane.

Whether the penetration of a molecule depends upon solution, adsorption, or chemical reaction the probability is strong that the cytoplasm itself actively participates in some manner in the movement of substances through it. For example, after molecules of a penetrating substance dissolve in, are adsorbed by, or combine chemically with micelles of the membrane, their penetration may be facilitated by kinetic activity of the membrane particles. A micelle might act in the capacity of a ferry boat, transporting a cargo of one or more molecules from one side of the membrane to the other. Once across the membrane many of the passenger molecules would escape because of their

relatively low concentration in the medium in contact with that side of the membrane. A seemingly more probable hypothesis is that the cargoes of adsorbed, dissolved, or chemically bound molecules are not carried all the way across the membrane by one micelle, but pass from particle to particle as impacts occur between them. Each micelle would, according to this hypothesis, carry a given molecule only for a very short portion of its journey across the membrane.

The Mechanism of Changes in Membrane Permeability.—The permeability of the cytoplasmic membranes fluctuates continuously as a normal feature of cellular activity. Some changes in the permeability of these membranes are induced by variations in factors of external origin as previously discussed, but others are due to unrecognized factors in the "internal environment" of the membranes. Furthermore, the living membrane of a given cell may at times be more permeable to some substances in certain regions than in others, whereas a short time later the distribution of the relatively permeable areas may be very different.

Various suggestions regarding the mechanisms by which changes in the permeability of the cytoplasmic membranes can take place have been proposed. Among these are: (1) changes in the viscosity of the membrane, (2) reversal of phase (on the assumption that the membrane has an emulsion-like structure), and (3) changes in the proportion of water in different phases of the colloidal system which is supposed to constitute the membrane. Since all of these hypotheses are highly speculative any detailed consideration of them in an introductory discussion would be unjustified.

Discussion Questions

1. How would you demonstrate that it is the cytoplasm of a living palisade cell that acts as a differentially permeable membrane and not the cell wall?
2. A decrease in temperature apparently decreases the permeability of the cytoplasmic membranes to water. What are some possible explanations?
3. If the tonoplast and plasmalemma act as differentially permeable membranes why does not the water in the vacuole move osmotically into the cytoplasm and cause the cytoplasm to displace much of the vacuole?
4. How can sudden changes be produced in the turgor of the root hairs of solution grown plants without injury to the cells?
5. What effect would an increase in the permeability to water of the cytoplasmic membranes of an algal cell immersed in pure water have upon the turgidity of the cell?
6. When algal cells are immersed in a strong glucose solution and similar cells in an ethylene glycol solution of equal molarity they plasmolyze (Chap. XI) rapidly, but recovery from plasmolysis occurs much more rapidly in the latter solution than in the former. What does this indicate regarding the permeability of the cytoplasmic membranes to these two compounds?

7. An algal filament is immersed in a dilute glycerol solution and the solution allowed to lose water by slow evaporation. Even when the solution has attained a concentration of about 50 per cent glycerol the filament shows no evidence of plasmolysis. Explain. If the filament is then transferred into pure water it swells and bursts almost immediately. Explain.
8. Why does NH_4OH apparently penetrate into many plant cells more readily than most other bases?
9. How do you account for the finding that when a NH_4Cl solution is injected into a plant cell the cell sap becomes more acid, but if the cell is immersed in a NH_4Cl solution the cell sap becomes more alkaline?
10. How would you determine whether or not the cytoplasm of a root hair cell is permeable to sucrose?
11. What evidence is there in support of the view that the tonoplast and plasmalemma are chiefly responsible for the differential permeability of the cytoplasm?
12. How could you demonstrate the effect of temperature variations upon the permeability of the protoplasm of a living cell to a certain solute within the range of 10-40° C.?

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CHAPTER XI

THE OSMOTIC QUANTITIES OF PLANT CELLS

The Plant Cell as an Osmotic System.—We have already become acquainted with the typical plant cell as a three dimensional structure, limited by cell walls; these walls in turn being lined with a layer of protoplasm which encloses a vacuole filled with cell sap. The protoplasm is normally held against the cell wall by a pressure exerted by the cell sap. The cell walls of most living cells are elastic within limits, but possess sufficient tensile strength so they do not commonly rupture. The cytoplasm, although usually possessing a high degree of fluidity, is usually much less elastic than the walls. The cell wall membranes of most living plant cells appear to be quite freely permeable to water and to most substances dissolved in it. The cytoplasmic membranes, on the contrary, almost invariably possess the property of differential permeability. Water and certain solutes pass through them much more readily than certain other solutes.

Plasmolysis.—If a plant cell which is at least partially distended is immersed in a hypertonic [1] solution a characteristic series of changes takes place in the appearance of that cell. The first detectable occurrence is a gradual shrinkage in the volume of the *entire cell* due to outward osmosis, and a consequent reduction in the pressure exerted by the cell sap against the protoplasm and cell wall. This shrinkage in volume can be detected in many cells by actual measurement under a microscope, although there are some types of plant cells in which little or no change in volume occurs under such conditions. There is a lower limit to the elasticity of the cell wall, however, and when this is attained no further contraction in the volume of the cell will occur. Since the cell wall is quite freely permeable to the water and the solutes of the surrounding solution, and hence the hypertonic solution is in contact with the outer surface of the protoplast, the cell sap will continue to lose water by outward osmosis, just as if no cell wall were present. Hence the protoplasm will continue to shrink in volume after the contraction of the

[1] With reference to a given solution, in this case the cell sap, a *hypertonic* solution is one with a higher osmotic pressure than the reference solution. Similarly an *isotonic* solution is one with an osmotic pressure equal to that of the reference solution, while a *hypotonic* solution is one of lesser osmotic pressure.

cell wall has ceased as, unlike the latter, the protoplasm has no lower limit of elasticity. At this point, therefore, the protoplasmic layer will begin to separate from the cell wall. If the hypertonic solution is strong enough this separation of the protoplasm from the cell wall will become very pronounced. In some types of cells the protoplasm will "ball up" into a more or less spherical mass within the cell. More often the shrunken protoplasm assumes other configurations as shown in Fig. 27. This phenomenon is called *plasmolysis*. The pattern followed by the shrinkage of the protoplasm in plasmolysis is more or less typical for each kind of cell, although it may be modified

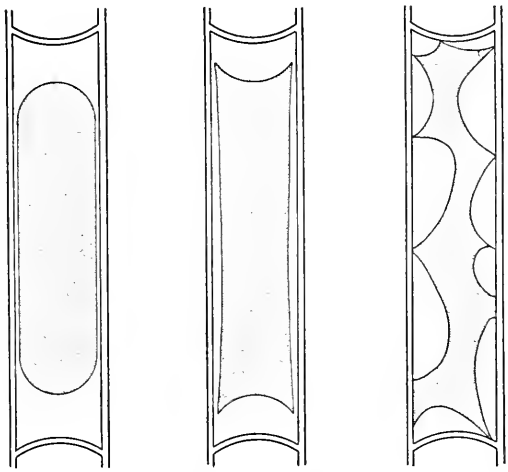

FIG. 27. Several common types of plasmolysis (diagrammatic).

somewhat depending upon physico-chemical conditions within the protoplasm, kind of solute used in the plasmolyzing solution, etc. The space between the cell wall and the protoplasm will be filled, after the separation of the latter from the wall, with the external solution.

If a plasmolyzed cell is immersed in water it will slowly recover and regain a turgid state, due to osmotic movement of water into the cell sap. Similarly if immersed in a solution hypotonic to the cell sap recovery will also ensue, but the degree of turgidity attained will be less than if the cell is immersed in pure water. Rapid "deplasmolysis," however, results in the death of many kinds of plant cells (Iljin, 1934).

In the preceding discussion plant cells have been considered purely as osmotic systems and we will continue to so regard them throughout the chapter. Although this has been the prevailing concept—at least regarding

mature, thin-walled plant cells with prominent vacuoles—the possibility that other mechanisms may also operate in the cell-to-cell movement of water should not be disregarded. The cytoplasm may participate actively in the passage of water into or out of plant cells. Bennett-Clark, *et al.* (1936) present evidence which they believe indicates that cytoplasm may "secrete" water into the vacuoles of some types of cells. Electro-osmotic phenomena (Chap. VIII) may also be involved in the cell-to-cell movement of water. Furthermore, in some types of cells the vacuoles are very small or are entirely filled with mucilaginous materials of one type or another. In such cells imbibitional phenomena probably play a greater proportionate role in the movement of water than in cells in which the vacuole occupies most of the cell volume.

Methods of Determining the Osmotic Pressures of Plant Cells and Tissues.—The *plasmolytic* method and the *cryoscopic* method are in common use for determining the osmotic pressure of plant cells and tissues. Both methods have many limitations and neither is as accurate as might be desired.

DeVries (1884) was the first to use the plasmolytic method (Chap. VIII) which in principle is very simple. A series of solutions (usually of sucrose) graded according to volume molar concentration is first prepared. The range of concentrations to be used depends upon the tissue to be studied. Comparable strips or sections of the tissue are then immersed in each solution and left until an osmotic equilibrium is attained. After immersion in the solution the pieces of tissue are observed under a microscope. In the stronger solutions it will be found that all of the cells are severely plasmolyzed, while in the weaker ones little or no sign of plasmolysis can be detected. Somewhere in the series will be found a solution in which about one-half of the cells are not plasmolyzed, and about one-half are more or less completely plasmolyzed. The average osmotic pressure in the cells of the tissue under investigation is considered to be equal to the osmotic pressure of the solution in which this condition obtains.

The value obtained by the procedure just outlined is called the *osmotic pressure at incipient plasmolysis.* This value is often greater than the true osmotic pressure of the cells since plasmolysis of many kinds of cells is preceded by a shrinkage in their total volume. A cell which has an osmotic pressure of 15 atmos. at incipient plasmolysis might have had an osmotic pressure of (for example) 12 or 13 atmos. in its normally distended state.

Certain more precise methods of determining the osmotic pressure of cells by the plasmolytic method, which take into consideration their volume changes, have also been devised. If incipient plasmolysis is determined to occur for cells of a certain tissue in a sucrose solution with a volume molar concentra-

tion of M_i, then the volume molar concentration of sucrose equivalent to that of the cell sap at its original volume M (*i.e.* before the shrinkage due to plasmolysis), is related to M_i by the following equation:

$$M = \frac{M_i V_i}{V}$$

in which V and V_i represent the volume of the cell in its original condition, and at incipient plasmolysis respectively. Having determined M_i the corresponding molarity for the cell sap of the "normal" cell can be readily calculated.

The practical difficulty of all such methods is in measuring accurately the volume changes which occur in cells during plasmolysis. The usual practice is to measure the change in the length (sometimes also in the breadth) and to assume that such measurements are proportional to the change in volume of the cell. For some types of cells this assumption is probably valid, but with others it may result in serious errors.

Formerly solutions of a number of different compounds, including certain electrolytes, were used in plasmolytic determinations, but at the present time sucrose solutions are almost universally employed. They are preferred because: (1) sucrose is apparently non-toxic to the protoplasm, (2) it does not penetrate into plant cells at a very appreciable rate (Höfler, 1926), (3) it apparently has little or no influence on membrane permeability, and (4) the exact osmotic pressures of different molar concentrations of sucrose are known with greater accuracy than for most other solutions.

Even when sucrose is used as a plasmolyzing agent the method is subject to a number of errors, of which the following are the most serious: (1) An outward diffusion of solutes into the plasmolyzing solution may occur during the determination thus resulting in an apparent osmotic pressure which is less than the true one. (2) In many plant cells it is difficult to observe the initial stages in the separation of the protoplasm from the cell wall during plasmolysis. This is most easily observed in cells with a colored sap or in which the cytoplasm contains chloroplasts, hence they are generally used to demonstrate plasmolysis. (3) The cytoplasm sometimes adheres so strongly to the cell wall that a solution of considerably greater concentration than one just barely hypertonic to the cell sap may be required to bring about plasmolysis. As a result of this source of error the apparent osmotic pressure is greater than the true one. (4) If the osmotic pressure of the cells of organs of the higher plants is to be measured by this method, strips of tissue thin enough to permit its examination under a microscope must be cut out of the organ. The resultant mechanical shock to the cells may have profound influences on

conditions within them, which may in turn affect their osmotic pressures. (5) The time required for an equilibrium to be obtained between a cell and a solution which just causes incipient plasmolysis is often so great that various types of changes may take place in the cell during this period and render the determination of dubious accuracy (Ernest, 1935).

When other plasmolyzing agents than sucrose are used other sources of error are often introduced into the determination in addition to those listed above.

It is doubtful if measurements of osmotic pressure by the plasmolytic method are ever very accurate except perhaps for types of cells which are inherently well adapted to the method. The principal advantage of this method is, that with rare exceptions, it is the only feasible one available for measuring directly the osmotic pressures of living plant cells.

The principle of the cryoscopic method for determining osmotic pressures has already been described in Chap. VIII. Briefly, as usually applied to plants, the method consists in expressing the juice from a sample of plant tissue and determining its freezing point depression with a suitable thermometer [2] or a thermocouple system (Chap. XIV). The osmotic pressure can then be calculated from the freezing point depression by the usual equation.

It has been found to be impossible to obtain homogeneous samples of sap from plant tissues unless they are first treated in such a way as to kill the cells. Consequently the tissue is usually first subjected to freezing, heating, grinding, or exposure to toxic vapors before the sap is expressed.

Doubtless the expression under high pressure of the sap from tissues which have previously been subjected to such treatments has a modifying effect upon the properties of the expressed sap of most tissues as compared with the original cell sap. Furthermore the sap obtained represents a mixture of contributions from all of the cells—living and dead—in the tissues. At the best, determinations of osmotic pressure made on such saps can represent no more than an average of the osmotic pressures of all the cells in the tissue. In spite of the possibility of a considerable modification in the properties of the sap inherent in this method, there is probably a consistent correlation between values obtained in this way and the mean osmotic pressure of the cell sap of the cells in a tissue.

Magnitude of the Osmotic Pressures in Plant Cells.—In many discussions of the osmotic pressure of plant cells no distinction is made between the "osmotic pressure of the cells at incipient plasmolysis" and their osmotic pressure under more or less turgid conditions. When the term osmotic pressure is employed without qualification it can be assumed to refer to the osmo-

[2] A Haidenhain or Beckmann thermometer is generally used.

tic pressure of cells in their normally distended condition. It is generally considered that the results of determinations by the cryoscopic method represent approximately the osmotic pressure of the cells in this condition.

Different organs or tissues of the same plant may exhibit a wide range of osmotic pressures. Even similar organs on the same plant—leaves for example —may vary considerably among themselves in the average osmotic pressure of their cells. Furthermore the tissues within the same organ usually show a considerable variation in osmotic pressures. The mesophyll cells of most leaves, for example, show higher values than the epidermal cells.

In general the osmotic pressures of the plant cells and tissues [3] of the mesic species of North America vary in their extreme range from a fraction of an atmosphere to about 50 atmos. Most of the values lie, of course, within a narrower part of this range, probably within the limits of a 4 to 30 atmos. span of values. In general the cells of ligneous species of plants as a group appear to have somewhat higher osmotic pressures than herbaceous plants as a group. This generalization cannot of course be extended to a comparison of any individual ligneous species with an individual herbaceous species. The range of osmotic pressures in the leaf tissues of plants of the eastern United States is illustrated by the data in Table 18, which is self-explanatory. It should be clearly understood that values such as those given in this table by no means represent constants. Considerable variation may be found in any one species depending upon environmental conditions, position of leaf on the plant, etc.

Factors Determining the Osmotic Pressures of Plant Cells.—Any factor which influences either the water content of a plant cell or the solute content of its cell sap will have an effect on the magnitude of the osmotic pressure of that plant cell. The water content of the plant as a whole, and hence of its constituent cells, is controlled principally by the relative rates of transpiration and the absorption of water (Chap. XVIII). The latter process is influenced very markedly by the water content and other conditions prevailing in the soil. Individuals of the same species invariably have a higher osmotic pressure when growing under drought conditions than when provided with a favorable water supply. This is at least partially due to the low water content of the leaves which results when the available soil water supply becomes low. Other factors which are also probably involved are a decrease in the growth rate of the plant which often permits an accumulation of mineral salts and soluble foods, and a shift of the starch $\rightleftarrows$ soluble carbohydrates

[3] It should perhaps be emphasized that the term "osmotic pressure of a tissue" can possess meaning only in the sense of the average osmotic pressure of the cells composing that tissue.

TABLE 18—OSMOTIC PRESSURES OF THE LEAVES OF VARIOUS SPECIES
(DATA OF HARRIS, 1934, AND MEYER, 1927)

Species		Osmotic pressure (atmos.)
Ligneous species		
Black Locust	*Robinia pseudacacia*	12.6
Black Walnut	*Juglans nigra*	14.6
Cottonwood	*Populus deltoides*	21.3
Dogwood	*Cornus florida*	17.3
Loblolly Pine (summer)	*Pinus taeda*	18.4
Mountain Laurel	*Kalmia latifolia*	18.1
Pitch Pine (summer)	*Pinus rigida*	18.2
Red Maple	*Acer rubrum*	17.3
Sassafras	*Sassafras sassafras*	20.4
Scarlet Oak	*Quercus coccinea*	19.1
Sweet Gum	*Liquidambar styraciflua*	15.5
Sycamore	*Platanus occidentalis*	12.1
Tree-of-Heaven	*Ailanthus glandulosa*	16.9
Tulip Tree	*Liriodendron tulipifera*	16.0
White Ash	*Fraxinus americana*	16.4
White Oak	*Quercus alba*	20.4
Willow	*Salix alba*	11.4
Yellow Birch	*Betula lutea*	15.1
Herbaceous species		
Blue Grass	*Poa pratensis*	13.6
Burdock	*Arctium minus*	10.8
Cat-tail	*Typha latifolia*	11.8
Chickweed	*Alsine media*	7.3
Cinnamon Fern	*Osmunda cinnamomea*	10.7
Cocklebur	*Xanthium sp.*	11.6
Dandelion	*Taraxacum taraxacum*	13.6
Jack-in-the-Pulpit	*Arisaema triphyllum*	8.5
Mullein	*Verbascum thapsus*	13.4
Patience Dock	*Rumex patientia*	6.7
Pokeweed	*Phytolacca decandra*	8.7
Soapwort	*Saponaria officinalis*	11.7
Sunflower	*Helianthus annuus*	18.8
Touch-me-not	*Impatiens biflora*	7.6
Wandering Jew	*Zebrina pendula*	4.8
Water Lily	*Castalia odorata*	14.7

equilibrium towards the side of the soluble carbohydrates (Chap. XXII). The effects of different soil water contents on the osmotic pressure of the tops and roots of maize plants are illustrated in Table 19.

TABLE 19—THE EFFECTS OF DIFFERENT SOIL WATER CONTENTS UPON THE OSMOTIC PRESSURE OF MAIZE PLANTS (DATA OF HIBBARD AND HARRINGTON, 1916)

Water content of soil. Per cent dry weight	Osmotic pressure of tops	Osmotic pressure of roots
31 per cent	22.06 atmos.	5.91 atmos.
23	23.08	7.23
16	24.36	7.79
14	25.04	9.24
13	25.47	11.34
11	26.48	11.98

The solute content of the cell sap is controlled by the specific metabolic processes of the plant and by the absorption of mineral salts by the plant from its environment. The rate of photosynthesis is an important factor in determining the osmotic pressure of plant cells, particularly those of leaf tissues. The influence of the inherent metabolic processes of any species upon the kinds and concentrations of various types of soluble organic compounds produced, such as simple carbohydrates, organic acids, amino acids, etc. has an exceedingly important effect on the magnitude of the osmotic pressure in any species. Metabolic conditions and their effects upon osmotic pressures may also be altered by environmental conditions. A well-known example of this is the difference in the osmotic pressures of sun and shade leaves on the same plant. The former almost invariably have the higher osmotic pressure, due presumably to their greater photosynthetic activity.

The mineral salts which contribute to the osmotic pressures of plant cells are all absorbed from the soil solution, or in aquatic species from the water in which part or all of the plant is immersed. Different species of plants vary greatly in their toleration of high concentrations of mineral salts in the soil. All species can become adjusted within limits to a change in the mineral salt content of the substratum. This adjustment takes the form of an increase in the osmotic pressure of the plant with an increase in the osmotic pressure of the medium from which it obtains its mineral salts (Table 20).

Other tissues of plants also show an increase in osmotic pressure with increase in the osmotic pressure of the soil solution, but such increases are generally most pronounced in the roots.

Species indigenous to alkali soil regions usually have relatively high osmotic pressures. Alkali soils are rich in soluble salts, and the high osmotic pressures found in species native to such soils are due to the absorption of relatively large quantities of mineral salts. In fact the highest recorded os-

TABLE 20—THE EFFECT OF THE OSMOTIC PRESSURE OF THE SOIL SOLUTION UPON THE OSMOTIC PRESSURE OF THE ROOTS OF MAIZE (DATA OF MC COOL AND MILLAR, 1917)

Osmotic pressure of soil solution	Osmotic pressure of sap from root cells
1.21 atmos.	4.59 atmos.
1.99	5.48
3.38	6.61
4.96	7.51
7.22	8.19

motic pressure for any species of plant—202.5 atmos.—was found in an alkali soil species, *Atriplex confertifolia* (Harris, 1934). Salt marsh plants also generally have relatively high osmotic pressures. This is also a correlation with the relatively high salt content of the substratum.

Diurnal Variations in the Osmotic Pressures of Plant Tissues.—The osmotic pressures of plant tissues fluctuate diurnally. Such variations are

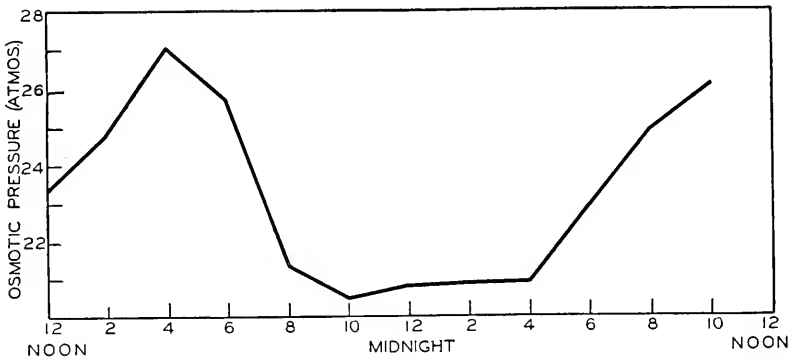

FIG. 28. Daily variation in the osmotic pressure of the leaves of *Andropogon scoparius*. Data of Stoddart (1935).

more marked in the aerial than in underground portions of plants. Their magnitude and direction are very strongly influenced by the environmental conditions to which the plant is subjected. Daily variations in osmotic pressure have been studied more extensively in the leaves than in any other organ of the plant. The data for the leaves of the prairie grass, *Andropogon scoparius* (Fig. 28), were obtained under extreme drought conditions, but similar, although less marked diurnal variations in the osmotic pressure of leaves are undoubtedly of regular occurrence in most species, at least on clear days.

The rather consistent increase in the osmotic pressure of leaves which usually occurs during the daylight hours is probably conditioned primarily by two factors: the accumulation of soluble carbohydrates or other organic compounds resulting directly or indirectly from photosynthesis, and the decrease in the water content of the cells due to an excess rate of transpiration over the rate of absorption of water resulting in a shrinkage in the volume of the cells. The minimum leaf osmotic pressure, which is usually attained between midnight and dawn, probably corresponds to the period when the leaf cells are at their maximum water content, and at their minimum organic solute content, due to the continuance of translocation out of the leaves during the night.

Seasonal Variations in the Osmotic Pressures of Plant Tissues.— Pronounced seasonal changes also occur in the osmotic pressures of plant

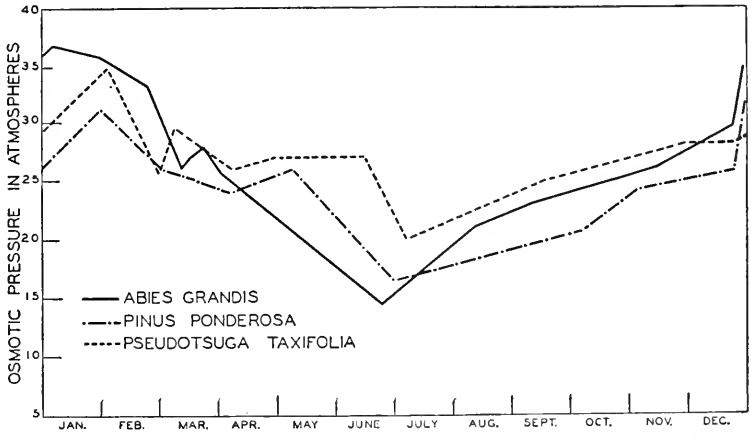

FIG. 29. Seasonal variations in the osmotic pressure of the leaves of evergreens. Data of Gail (1926).

tissues. In temperate regions the osmotic pressures of at least the exposed organs of plants are usually higher in the winter than in the summer. Seasonal variations in the osmotic pressures of temperate zone plants have been studied principally in the leaves of evergreen species (Fig. 29). Such variations are also known to occur in the exposed woody stems of plants, although information about them is much less complete. The increased winter osmotic pressures are undoubtedly correlated principally with an increase in the soluble carbohydrates in the cell sap due to a shift in the starch $\rightleftarrows$ soluble carbohydrate equilibrium caused by low temperatures (Chap. XXII) and to a lesser extent to the reduced water content of the leaf tissues which is usually characteristic of the winter condition. In some species the first of these factors is

predominant, in other species the second (Steiner, 1933). Similar seasonal variations in osmotic pressure occur in the leaves of evergreen herbaceous species, as for example winter wheat.

In many woody species of semi-desert regions of the southwestern United States and adjacent Mexico the osmotic pressure of the leaves increases markedly during the hot, dry season (May-July) but shows a rapid decrease with

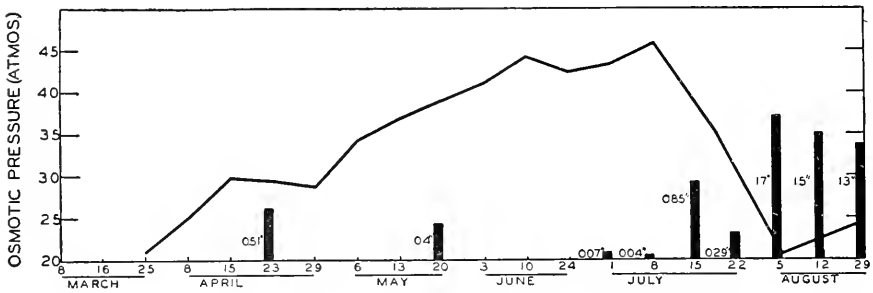

FIG. 30. Seasonal variation in the osmotic pressure of the leaves of the creosote bush (*Larrea tridentata*) under desert conditions. Height of black columns indicates the rainfall in inches. Data of Mallery (1935).

the advent of summer rains (Fig. 30). The increasing osmotic pressure of the leaves of such species during the dry months is closely correlated with the increasing unavailability of soil water.

The Osmotic Quantities of Plant Cells.—Let us suppose three similar cells, each in a state of incipient plasmolysis, and each with a cell sap osmotic pressure of 10 atmos., to be immersed, the first in pure water, the second in

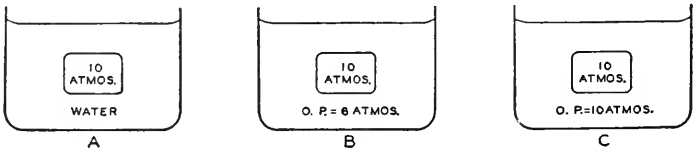

FIG. 31. Similar cells immersed in solutions of different osmotic pressures.

a solution with an osmotic pressure of 6 atmos. and the third in a solution with an osmotic pressure of 10 atmos. (Fig. 31). The volume of the liquid in which each cell is immersed is supposed to be very large in proportion to the volume of the cell.

In order to simplify the discussion certain assumptions will be made regarding these cells and, *unless stated to the contrary, regarding all other cells*

discussed in the remainder of this chapter. These are: (1) that the cells are at the same temperature, (2) that the cytoplasmic membranes are permeable only to water while the cell walls are freely permeable to both water and solutes and (3) that the walls of all the cells are equally elastic. Furthermore, changes in the osmotic pressure of the cell sap of these three cells due to the changes in the volume of the cells will be disregarded.

Diffusion of water into cell *A* begins immediately upon its immersion. This inward movement of water is osmosis. The diffusion pressure deficit of the water in the cell sap is 10 atmos. (since its osmotic pressure is 10 atmos., and it is not subjected to pressure); that of the surrounding water zero, hence the diffusion pressure of the external pure water is 10 atmos. greater than that of the water in the cell sap. The entrance of water into the cell exerts a gradually increasing pressure against the protoplasm which is in turn transmitted to the cell wall. This pressure is called the *turgor pressure* (*cf.* discussion in Chap. VIII) and is the cause of the gradual distension of the cell. As soon as any turgor pressure is exerted against the walls of the cell they exert a counter pressure—the *wall pressure*—against the protoplasm and cell sap. The wall pressure is always equal to but acts in the opposite direction to the turgor pressure. The maximum turgor pressure which can be developed by this particular cell is 10 atmos., since, as already shown in Chap. VIII, the osmotic pressure of a solution is a measure of the maximum turgor pressure which it can develop. At this point the wall pressure of the cell will also be equal to 10 atmos. The wall pressure of a cell will have exactly the same effect upon the diffusion pressure of the water in the cell sap that a pressure of equal magnitude exerted by a piston would have on water subjected to it (Chap. VIII). In this cell, therefore, the wall pressure will increase the diffusion pressure of the enclosed cell sap by 10 atmos. Since the initial diffusion pressure deficit of the cell sap was 10 atmos. and the development of a wall pressure of 10 atmos. has raised the diffusion pressure of the water in the cell sap by 10 atmos. the diffusion pressure deficit of the water in the cell sap is reduced to zero. Since this is also the diffusion pressure deficit of the surrounding water, a dynamic equilibrium has been established due to the influence of the wall pressure upon the diffusion pressure of the water in the cell sap. When this condition of dynamic equilibrium has been attained, equal numbers of water molecules will be passing across the membrane in both directions per unit of time.

It is the turgor pressure of the cell acting in opposition to its wall pressure which imparts to plant cells their usual rigid, distended condition. This condition is termed *turgor, turgidity*, or *turgescence;* the first of these terms being in most general use. Plant cells, although generally turgid, seldom

attain a turgor pressure equal to their osmotic pressure. Cells low or entirely lacking in turgor pressure are usually referred to as *flaccid*.

Cell *C* will remain, according to the conditions prescribed for this experiment, in a state of incipient plasmolysis. The diffusion pressure deficit of the water in the cell sap and in the solution are equal (*i.e.* their osmotic pressures are equal and neither is under any pressure, except, of course, atmospheric pressure) hence a dynamic equilibrium will be established as soon as the cell is immersed in the solution. Since there is no net movement of water into the cell no turgor pressure and hence no wall pressure will develop, a condition which will obtain in all plant cells in the condition of incipient plasmolysis.

The diffusion pressure deficit of the water in the cell sap of cell *B* is 10 atmos., that of the water in the surrounding solution 6 atmos. Hence the diffusion pressure of the water in the solution is 4 atmos. greater than that of the water in the cell sap, and there will be a net inward movement of water into the cell. A turgor pressure thus develops within the cell, but at its maximum under these conditions it will attain a value of only 4 atmos. Counterbalancing this turgor pressure will be a wall pressure of 4 atmos. Since the initial diffusion pressure deficit of the water in the cell sap was 10 atmos., imposition of a wall pressure of 4 atmos. reduces this to 6 atmos., which is the diffusion pressure deficit (osmotic pressure) of the water in the surrounding solution. Hence the attainment of a turgor pressure of only 4 atmos. results under these conditions in an osmotic equilibrium. Since the magnitude of the turgor pressure developed in cell *B* will be less than in cell *A*, the latter cell will be more completely distended than the former.

In all three of these cells a dynamic equilibrium has been attained by an adjustment of the diffusion pressure deficit of the water in the cell sap until it is equal to that of the water in the surrounding liquid. This adjustment was attained in each of these cells by a shift in the magnitude of the wall pressure, which is one of the two components determining the diffusion pressure deficit of the water in the cell sap, the other being the osmotic pressure. Unlike a solution exposed to the atmosphere, the diffusion pressure deficit of a cell is not equal to its osmotic pressure except when the cell is flaccid, *i.e.*, possesses a zero wall pressure. The term "diffusion pressure deficit of a cell" should be considered as an abbreviation for "diffusion pressure deficit of the water in the cell sap."

The inter-relationships among the osmotic pressure, turgor pressure, wall pressure, and diffusion pressure deficit of a plant cell should be further clarified by a study of Fig. 32. This diagram also takes into account the influence of volume changes in the cell upon these physical quantities. In the interests

of simplicity the effects of such volume changes upon the osmotic pressure, etc. of the cell have been disregarded in the discussion up to this point. It is assumed that the wall pressure of the cell increases proportionately with an increase in the volume of the cell. When the cell is completely flaccid (relative volume = 1.0) its diffusion pressure deficit is equal to its osmotic pressure (20 atmos.) while its turgor pressure and hence its wall pressure are both zero. As the volume of the cell increases, due to an influx of water, the osmotic pressure decreases due to dilution of the cell sap. The turgor pressure and wall pressure increase equally and progressively with increase in the volume of the cell, the diffusion pressure deficit of the cell showing a corre-

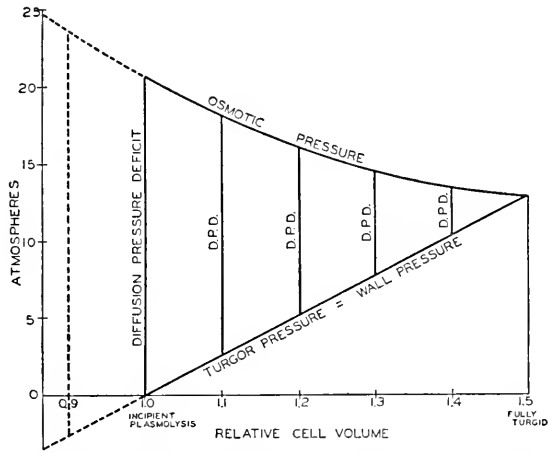

FIG. 32. Inter-relationships among the osmotic pressure, turgor pressure, wall pressure, diffusion pressure deficit, and cell volume of a plant cell. Modified from Höfler (1920).

sponding but more rapid, progressive decrease. When the cell attains a condition of maximum turgidity (relative volume = 1.5) its turgor pressure and wall pressure are equal to its osmotic pressure while its diffusion pressure deficit has fallen to zero.

The dotted extension of the line for wall pressure to the left is intended to suggest that it sometimes has a negative value. Conditions under which this may occur are discussed shortly. The diffusion pressure deficit of a cell in which the wall pressure is negative is greater than its osmotic pressure.

In many types of cells volume changes are much less marked than indicated in this figure, and hence they can often be disregarded in generalized considerations of the water relations of plant cells. In some species many of the cells have walls which are totally lacking in elasticity. This is especially

true of cells in the tissues of xerophytes and some water plants (Ernest, 1934 b).

The fundamental relations between the osmotic pressure, wall pressure, turgor pressure, and diffusion pressure deficit of a plant cell are expressed in the following simple equations:

$$\text{Diffusion pressure deficit} = \text{Osmotic pressure} - \text{Wall pressure}$$
$$\text{Wall pressure} = \text{Turgor pressure}$$

For example, cell *B* (Fig. 31) after attaining an equilibrium with a solution of 6 atmos. osmotic pressure, had an osmotic pressure of 10 atmos., and a wall pressure of 4 atmos. Hence its diffusion pressure deficit was 6 atmos. and its turgor pressure was 4 atmos.

The quantity to which the name "diffusion pressure deficit" has been given in this discussion has been variously termed by writers in the field of plant physiology (Ursprung, 1935). Among the terms which have been used are "suction," "suction force," "suction pressure," "suction tension," "water-absorbing power," "turgor deficit," and "net osmotic pressure." All of these terms are likely to be encountered in any extensive reading upon the subject of the water relations of plants. The authors of this book are not convinced that any of these terms is entirely free from criticism nor that any has as yet gained the sanction of even approximately universal usage. Hence we shall adhere to the term "diffusion pressure deficit" as a name for this physical quantity throughout this book. Those who have a decided preference for any other term need only to substitute it for "diffusion pressure deficit" wherever it occurs in the text.

The osmotic pressure, diffusion pressure deficit, turgor pressure, and wall pressure are collectively called the *osmotic quantities* of plant cells. As previous discussion has shown a full evaluation of these quantities also requires a consideration of the volume changes of plant cells.

In plant tissues many of the cells are under a pressure imposed upon them by the surrounding cells. In addition to its own wall pressure the water in such a cell is also subjected to this pressure of external origin. This pressure is just as much a factor in determining the diffusion pressure deficit of a cell as its own wall pressure. The true diffusion pressure deficit of such a cell is therefore less than that of the cell considered as an individual unit by the amount of this added pressure. Hence the equation representing the factors determining the diffusion pressure deficit of a cell under such conditions becomes:

$$\text{Diffusion pressure deficit} = \text{Osmotic pressure} - \text{Wall pressure} - \text{Pressure exerted by surrounding cells.}$$

The water in the vessels and cells of plants frequently passes into a state of tension. Imposition of pressure from an external source raises the diffusion pressure of water. Imposition of a tension (which is equivalent to a negative pressure) has precisely the opposite effect. In cells and vessels this can only happen if the enclosed water contracts in volume to such a point that the encompassing walls are pulled inwards due to adhesion between the water and the walls. The counter pull exerted by the walls on the water results in throwing it into a state of tension. Under such conditions the wall pressure and hence the turgor pressure of the cell are negative in value (Fig. 32), and the tension ("negative pressure") developed within the water will be equal in magnitude to the negative wall pressure. The equation for the diffusion pressure deficit of a cell under such conditions becomes:

$$\text{Diffusion pressure deficit} = \text{Osmotic pressure} - (-\text{Wall pressure})$$

In other words the diffusion pressure deficit of the water in a cell or vessel when subjected to a tension is equal to the osmotic pressure plus the tension imposed on the water.

The diffusion pressure deficit of any plant cell, if we disregard for the time being the possible effect of tensions, may range in value from zero to the osmotic pressure of that cell when flaccid. The range of diffusion pressure deficits in plant tissues therefore corresponds very closely with the range of osmotic pressures, as discussed earlier in this chapter.

Dynamics of the Intercellular Movement of Water in Plants.—In order to simplify this part of the discussion changes in the osmotic pressure of cells due solely to volume changes (as shown in Fig. 32) will again be disregarded, as usually these are not great enough to modify seriously any generalized picture of the water relations of plant cells.

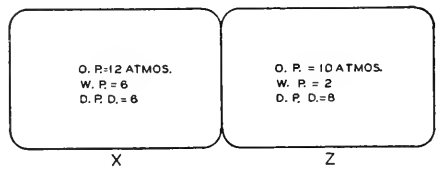

FIG. 33. Diagram of two adjacent cells used in explanation of the mechanism of cell-to-cell movement of water.

Let us imagine a certain cell (X) to have an osmotic pressure of 12 atmos., and a wall pressure of 6 atmos.; and a second cell (Z) to have an osmotic pressure of 10 atmos., and a wall pressure of 2 atmos. Let us further suppose that these two cells are brought into such intimate contact that a normal osmotic movement of water can occur between them, under conditions that no evaporation can occur from them (Fig. 33).

Which cell will gain water under these conditions? An uncritical interpretation would answer that water will move from cell Z to cell X, since

the cell sap of X has the higher osmotic pressure. Although such an explanation is commonly offered, even in some textbooks, a little consideration shows that this cannot possibly be true.

The water in the cell sap of X would have a diffusion pressure deficit of 12 atmos. were it under no pressure. The wall pressure of 6 atmos., however, reduces the diffusion pressure deficit of the cell to 6 atmos. Similarly the water in the vacuole of cell Z would have a diffusion pressure deficit of 10 atmos. were it not also influenced by a wall pressure of 2 atmos. Hence the diffusion pressure deficit of cell Z is 8 atmos. The diffusion pressure deficit of Cell X is therefore less than that of cell Z. Water will therefore move from cell X to cell Z. Water will continue to show a net movement from X to Z until the diffusion pressure deficits of the two cells are equal. In the movement of water from cell to cell in plants it is the diffusion pressure deficits and not the osmotic pressures which tend to equilibrate. This is only a special aspect of the fundamental tendency of the diffusion pressure of water to attain a uniform value throughout any system. It is by no means impossible, therefore, for water to move from a cell of higher to one of lower osmotic pressure, although it is not to be inferred that this is generally or even usually true. Any cell will "absorb" water from a cell or solution of lower diffusion pressure deficit and, correspondingly, will lose water to a cell or solution of higher diffusion pressure deficit.

It should not be supposed that after a dynamic equilibrium has been established between two cells that their diffusion pressure deficits will be an exact average of their initial diffusion pressure deficits, as this is seldom true. Differences in the original volume of the cells and the elasticity of the cell walls usually make this impossible. The only general statement which can be made is that, at equilibrium, the diffusion pressure deficits of the two cells will be equal, and this value will lie somewhere between the two original values of the cells.

Whenever the diffusion pressure deficits of two adjacent cells are dissimilar we may speak of a *diffusion pressure deficit gradient* as existing between them. Movement of water from one cell to another can occur only when such a gradient exists. Other conditions being equal the "steeper" this gradient, *i.e.* the greater the difference in diffusion pressure deficits, the more rapidly one cell will gain water from the other. The term diffusion pressure deficit gradient can also be applied to a chain of cells in which the diffusion pressure deficit increases serially from cell to cell. Several examples of such gradients will be discussed in subsequent chapters.

Water Relations within Individual Plant Cells.—Imbibition of water is due fundamentally to a lesser diffusion pressure of water in the imbibing

substance than in the surrounding water (Chap. IX). We may therefore also speak of the diffusion pressure deficit of an imbibing substance. A block of dry gelatin, for example, dropped into a solution with an osmotic pressure of 10 atmos. will, after a dynamic equilibrium has been established, have a diffusion pressure deficit of 10 atmos. A completely saturated imbibant will have a diffusion pressure deficit of zero. The development of what we have just called the diffusion pressure deficit of an imbibing substance is a more complicated phenomenon than the development of the diffusion pressure deficit of a solution, but further details cannot be considered here.

Within a plant cell the cell sap, the water in the protoplasm, and the water in the cell wall may each be regarded as possessing a diffusion pressure deficit of its own. The diffusion pressure deficit of the protoplasm and the cell wall are at least in part of imbibitional origin. In an isolated plant cell from which evaporation of water is prevented the diffusion pressure deficits of all parts of a cell are usually approximately in equilibrium. If immersed in a solution all parts of a cell will more or less rapidly attain an equilibrium, not only with each other but with the surrounding solution.

If the magnitude of the diffusion pressure deficit of any part of a cell is either increased or decreased the values for other parts of the cell tend towards equilibrium with this new value. For example, if the diffusion pressure deficit of the mesophyll walls of a cell is increased due to evaporation from them during transpirational water loss, water will diffuse toward the walls from the protoplasm and cell sap as long as their diffusion pressure deficit is less than that of the walls. Similarly if the osmotic pressure of the cell sap is increased by a conversion of insoluble starch to sugars, water will diffuse into the cell sap from the wall and protoplasm until a diffusion pressure deficit equilibrium is attained within the cell.

Methods of Measuring the Diffusion Pressure Deficit of Plant Cells and Tissues.—By the methods at present in use it is possible to measure the diffusion pressure deficit only for cells with elastic walls. Most methods of measuring this quantity are based on the principle, previously discussed, that if a cell is immersed in a solution with an osmotic pressure equal to the diffusion pressure deficit of the cell, a dynamic equilibrium is immediately established, and no change will occur in the volume of the cell. In solutions with a higher osmotic pressure than the diffusion pressure deficit of the cell, it will decrease in volume; in solutions of lower osmotic pressure than its diffusion pressure deficit the cell will increase in volume.

The first step in making determinations of the diffusion pressure deficits of plant cells is to prepare a series of sucrose solutions graded at appropriate intervals in osmotic pressure. The range of the series to be used will depend

upon the possible diffusion pressure deficits in the material under investigation. Some investigators have undertaken to measure the diffusion pressure deficit of individual cells (Ursprung and Blum, 1916). Single cells or groups of such cells are isolated from the tissue, and mounted under the microscope, usually in paraffin oil (to prevent changes in diffusion pressure deficit due to evaporation, or osmotic movement of water into or out of the cells from or into an aqueous medium). The linear dimensions of the cells are recorded, after which a sample of the cells is immersed in each sucrose solution in the series. After the attainment of an equilibrium between the cells and the solutions they are remeasured under the microscope. The osmotic pressure of the solution in which the cells show no change in dimensions is considered to be equal to the diffusion pressure deficit of the cell. This method requires technique of considerable precision and has not been widely used.

A "simplified method" of measuring diffusion pressure deficits has also been introduced by Ursprung and Blum (1923). In this method narrow strips of tissue are cut from such structures as thin leaves or petals. The length of each strip is immediately measured under the microscope, usually while mounted in paraffin oil. Several strips are then immersed in each of a graded series of sucrose solutions in which they are allowed to remain until an equilibrium has been attained, after which they are remeasured. The osmotic pressure of the solution in which no change in the length of the strips occurs is considered to be equal to the average diffusion pressure deficit of the cells in the strip.

For the measurement of the average diffusion pressure deficit of the cells in bulky tissues such as potato tubers, masses of tissue such as cylinders of approximately equal dimensions can be employed. If possible all of the cylinders used in a given determination should be cut from the same organ. The equilibrium point can be determined by measuring changes either in the weight or the volume of the cylinders. The solution in which the cylinder neither gains nor loses weight (or volume) is considered to have an osmotic pressure equal to the average diffusion pressure deficit of the cells in the cylinder.

Determinations of diffusion pressure deficits by the methods described above should not be confused with plasmolytic determinations of the osmotic pressures of plant cells. In the latter type of determination the critical measurement is the osmotic pressure of the solution with which the cells come to an equilibrium without any turgor pressure, *i.e.* at incipient plasmolysis. In diffusion pressure deficit determinations the critical measurement is the osmotic pressure of the solution with which the cells come to equilibrium without any change in their turgor pressure, *i.e.* without any change in the volume of the cells. Only when the cell is initially in a completely flaccid

condition will determinations of its osmotic pressure and its diffusion pressure deficit result in the same values.

All of the methods of measuring the diffusion pressure deficits of plant cells are subject to most of the flaws which are also inherent in plasmolytic determinations of osmotic pressure, as well as some other serious errors (Ernest, 1931, 1934a). Except possibly for a few types of cells which are especially well adapted to such measurements, it is doubtful if the values obtained in determinations of diffusion pressure deficits are ever more than fair approximations of the true values which obtain in plant cells.

Neither the turgor pressure nor the wall pressure of a cell can be determined directly. However, if the osmotic pressure of a cell or group of cells has been determined, and likewise the diffusion pressure deficit, the turgor pressure or wall pressure can be calculated from the equations given earlier in the chapter.

Discussion Questions

Note: In all questions on diffusion pressure deficit ("suction tension") assume that membranes are permeable to water only, and that no changes occur in the osmotic pressure of the cells as a result of volume changes or other causes.

1. Can a cell with an osmotic pressure of 10 atmos. ever obtain water from a cell with an osmotic pressure of 12 atmos.? Explain.
2. Cell A has an osmotic pressure of 12 atmos. and is immersed in a solution with an osmotic pressure of 6 atmos. Cell B has an osmotic pressure of 10 atmos. and is immersed in a solution with an osmotic pressure of 8 atmos. Assume both cells are first allowed to come to equilibrium with the solution in which each is immersed, and that they are then removed and brought into contact. Which direction will water move? Why?
3. When placed in a solution of 6 atmos. osmotic pressure, cell A decreased in size, while cell B increased. Cell A just plasmolyzed in a solution of 12 atmos. osmotic pressure while B just plasmolyzed in a solution of 9 atmos. osmotic pressure. Cell B regained its original volume in a solution of 8 atmos. osmotic pressure. If in their original condition the cells had been placed side by side, which would gain water from the other? If placed in distilled water while in their original condition which cell would gain water more rapidly? If placed in a solution of 7 atmos. osmotic pressure which way would water move with respect to each cell?
4. Suppose you were assigned the problem of determining the osmotic pressure and diffusion pressure deficit of the leaves on an oak tree at different distances from the ground. Describe exactly how you would proceed.
5. A cell with an osmotic pressure of 12 atmos. manifests three-fourths of its maximum turgidity. What is the diffusion pressure deficit of the cell?
6. Cells A, B, and C, having osmotic pressures of 6, 8, and 3 atmos. respectively, constitute a chain of three cells in the order named. A part of the lowest cell, C, dips into a solution with an osmotic pressure of 2 atmos. None of the other cells is in contact with the solution, which is large in volume in comparison with the cells. Evaporation from the cells is prevented. What will be the diffusion pressure deficit and turgor pressure of each cell at equilibrium?

7. Suppose that evaporation was occurring from the surface of cell A (question 6). How would your answer to the question differ?
8. If all three of the cells (question 6) were completely immersed in the solution, what would be the diffusion pressure deficit and turgor pressure of each at equilibrium?
9. If a small block of tissue cut from a potato tuber or any other bulky tissue be immersed in a solution with an osmotic pressure of 8 atmos. what will be the diffusion pressure deficit of the cells in the tissue after a dynamic equilibrium has been established? Any exceptions?
10. A chain of cells, each of which has an osmotic pressure of 8 atmos. is arranged so that one terminal cell dips in a solution with an osmotic pressure of 3 atmos. and the other in a solution with an osmotic pressure of 6 atmos. The volume of both of these solutions is very large in comparison to the size of the cells. Evaporation is prevented. Will any movement of water occur? Explain.
11. What effect will a change of starch to sugar in a cell have upon its diffusion pressure deficit? an increase in the permeability of the cell membranes to solutes? to water?
12. A cell has an osmotic pressure of 15 atmos. and water evaporates from it until the cell walls are pulled inwards enough that the enclosed water is subjected to a tension of 12 atmos. What is the diffusion pressure deficit and wall pressure of the cell?
13. Measurements of the diffusion pressure deficit of the epidermis of a leaf sometimes show it to be lower than the diffusion pressure deficit of adjacent mesophyll cells from which it must obtain water. What is a possible explanation of this finding?
14. The cells of a parasitic fungus were found to have a lower osmotic pressure than the cells of the host plant upon which it was growing. How could the fungus obtain water from the host?
15. Pollen grains of cotton germinate readily upon the stigma but burst rapidly if floated upon water or a dilute sugar solution. Explain.

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CHAPTER XII

THE LOSS OF WATER FROM PLANTS

It is commonplace knowledge that all plants require water for their existence and development and that most plants require it in considerable quantities. It is not so generally recognized, however, that in most species of plants an overwhelmingly large proportion of the water absorbed from the soil is lost by the plant into the atmosphere and takes no permanent part in its development or in its metabolic processes. The lack of this general realization is probably due to the fact that while water is supplied to and absorbed by plants in its familiar liquid form, by far the greater part of that lost escapes in the invisible form of water-vapor.

The loss of water-vapor from living plants is known as *transpiration.* Loss of water-vapor may take place from any part of a plant which is exposed to the air. This applies even to roots in contact with the soil atmosphere. Generally speaking, however, the leaves are the principal organs of transpiration. Most of the transpiration from leaves occurs through the stomates; this is termed *stomatal transpiration.* Smaller amounts of water-vapor are lost by direct evaporation from the epidermal cells through the cuticle; this is usually called *cuticular transpiration.* All aerial parts of plants lose some water by transpiration, although, due to the presence on some organs of superficial layers almost impervious to water, the rate of loss from most such organs is very low. Some of the transpiration from herbaceous stems, flower parts, fruits, etc. is of the cuticular type, but is small in amount. Most herbaceous stems, fruits, and flower parts bear stomates which permit the occurrence of stomatal transpiration from such organs. Loss of water-vapor also takes place through the lenticels of woody stems; this is usually called *lenticular transpiration.*

The Mechanics of Foliar Transpiration.—The subsequent discussion will deal almost entirely with transpiration from leaves, since, in most plants, the amount of water-vapor lost from other organs is comparatively small. The mechanics of foliar transpiration can be adequately discussed only in reference to the anatomy of the leaves from which it occurs (Fig. 34, Fig. 35, and Fig. 36). It should be recalled that the vacuoles of all of the living cells

of a leaf are filled with water, which also saturates the protoplasm and the cell walls, this water being supplied to the leaf cells through the water conducting tissues of the vascular bundles. Hence evaporation of water will occur from these wet cell walls into the internal atmosphere of the inter-

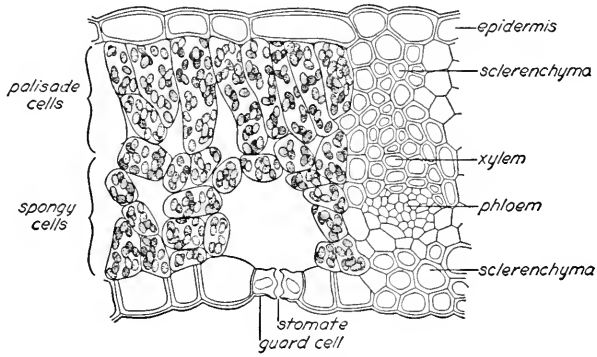

FIG. 34. Cross section of a portion of a leaf of tulip tree (*Liriodendron tulipifera*).

cellular spaces just as it will occur from any wet surface into the surrounding air. The intercellular spaces constitute a connected system, ramifying throughout the leaf. Under certain unusual conditions the intercellular spaces

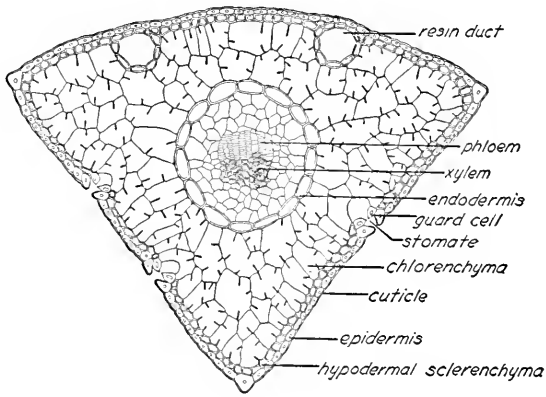

FIG. 35. Cross section of a leaf of white pine (*Pinus strobus*).

can become injected with liquid water but under all normal conditions they are occupied by air.

If the stomates are closed the only effect of evaporation from the mesophyll cell walls will be the saturation of the entire volume of the intercellular

spaces with water-vapor. When the stomates are open, however, diffusion of water-vapor may occur through them into the outside atmosphere. Such outward diffusion will always take place unless the atmosphere has a vapor pressure equal to or greater than that of the intercellular spaces, a condition

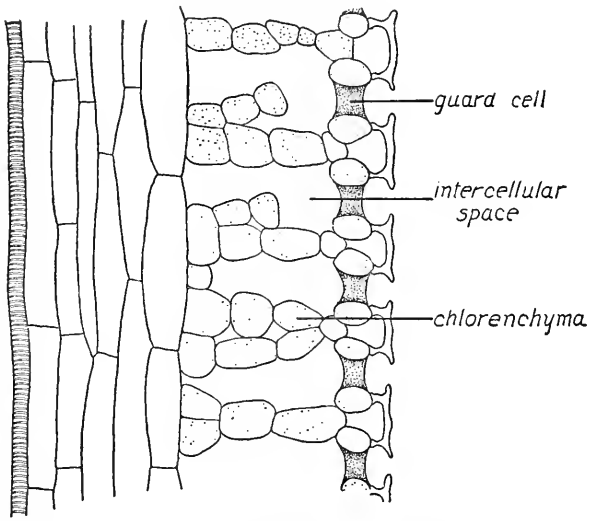

FIG. 36. Longitudinal section (semi-diagrammatic) through a small portion of a leaf of white pine (*Pinus strobus*).

which does not commonly exist during the daylight hours. The rate of such diffusion will depend principally upon the excess of the vapor pressure in the leaf over that of the atmosphere, although the "diffusive capacity" of the stomates (Chap. XIII) is also an important factor. The process of stomatal transpiration therefore involves *evaporation* from the cell wall surfaces bounding the intercellular spaces and the *diffusion* of this water-vapor from the intercellular spaces into the atmosphere through the stomates.

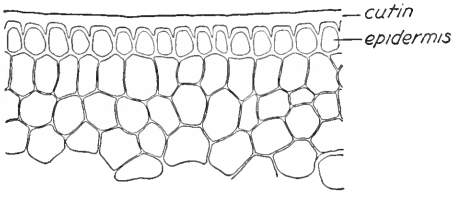

FIG. 37. Cutin layer on the upper epidermis of a leaf of *Clivia nobilis*.

One side of every epidermal cell on a leaf is also exposed to the atmosphere. Evaporation of water occurs into the atmosphere directly from these cell surfaces. The surfaces of practically all aerial leaves are covered with a layer of wax-like substance known as *cutin* (Fig. 37). This is not readily permeable to water and hence

reduces transpiration directly through the walls of the epidermal cells to a magnitude much less than it would be, were there no such layer present. The thickness of the cutin layer varies with the species of the plant, and the environmental conditions under which the leaves have developed. The layer of cutin is usually thicker on leaves which have developed in bright sunlight, for example, than on leaves of the same species which have developed in shade. Even in leaves which are heavily coated with cutin, some cuticular transpiration occurs, possibly largely through tiny rifts in the cutin layer. In most species of plants of the temperate zone less than 10 per cent of the foliar transpiration occurs through the cuticle, the remainder being stomatal transpiration.

Evaporation.—The fact that transpiration may be regarded as essentially a modified form of the process of evaporation makes desirable a fuller consideration of the dynamics of this process. When an open pan of water is exposed to the atmosphere, the level of the water in the pan slowly drops. Evidently water molecules are being slowly lost into the atmosphere. This is an example of the well known process of *evaporation*. All of the molecules in a mass of water are not travelling with the same velocity, although for any given temperature the *average* speed of all of the molecules in the liquid mass is a constant. Some of the molecules in the liquid water attain sufficient momentum to entirely overcome the attractive forces holding them in the liquid, and escape into the surrounding atmosphere in the form of vapor. The swiftest molecules present are most likely to be able to overcome the attraction of the other water molecules, therefore they are the most likely to be lost from the liquid. During evaporation any body of water is thus slowly being depleted of its more rapidly moving molecules. The residual water becomes progressively richer in relatively sluggish molecules; in other words it becomes cooler. This cooling effect is more or less completely offset, however, by physical transfer of heat into the water from its surroundings as soon as its temperature drops below that of the environment. The proportion of "high speed" molecules in the molecular population of the pan of water is thus maintained at close to its original value and the rate of evaporation continues with very little diminution.

In the preceding paragraph we have focussed our attention only on the water molecules which escape from the liquid. Water-vapor molecules are also returning to the liquid from the atmosphere during the process of evaporation. If a water-vapor molecule in the atmosphere above the evaporating surface, particularly one of the more sluggish ones, strikes the surface of the water at the proper angle and with not too great a velocity, it will be held there by the attractive forces exerted by the liquid water molecules, and again

become part of the liquid water. "High speed" molecules, on the other hand, are much less likely to be captured by the attractive forces exerted by the molecules at the surface of the body of liquid. They impinge upon the bounding film of the liquid with such velocity that unless they hit that surface at right angles or nearly so, they usually glance off along a new pathway.

This picture of the kinetics of the evaporation process holds for any evaporating surface whether it be the exposed surface of a body of water, a moist piece of cloth, paper, or porous clay, or the mesophyll cell walls of a leaf. Ordinarily the term evaporation is used to refer only to conditions in which the rate of escape of molecules exceeds their rate of return, and its use will be restricted to this sense in this discussion.

If the air above the water surface is confined, as for example, when a dish of water is covered by a bell jar, the number of water-vapor molecules in the confined space will gradually increase due to evaporation from the surface of the water. Since their movement is a random one the water-vapor molecules will be continually colliding with walls of the container, each other, the molecules of other gases present in the air, and surface of the liquid water. Some of those which strike the surface of the liquid will be held there by intermolecular attractive forces. As the concentration of water-vapor molecules in the air increases, the number which plunge back into the water in any unit interval of time will increase. Eventually a dynamic equilibrium will be attained at which the number of molecules leaving the surface and the number returning to it in a unit time will be equal. At this point the air will be *saturated* with water-vapor and evaporation will no longer be occurring.

The water-vapor molecules exert a definite pressure against the walls of the container and the surface of the water. This is known as *vapor pressure*. In the illustration given in the preceding paragraph, the vapor pressure of the water increases progressively until the saturation point is reached. The vapor pressure of water under the conditions of such a dynamic equilibrium may conveniently be termed the *saturation vapor pressure*. The vapor pressure of a liquid is usually expressed in terms of millimeters of mercury. The saturation vapor pressure of water at 20° C., for example, is 17.54 mm. Hg. This means that at this temperature the water-vapor exerts a pressure equal to the pressure exerted by a column of mercury 17.54 mm. high. The saturation vapor pressure of any liquid is independent of the area of the evaporating surface, but increases with increase in temperature (Table 23).

Botanists are often interested in measuring the rate of evaporation under a given set of conditions in connection with studies of transpiration and of plant distribution. The difficulties involved in making this apparently simple

measurement are not all apparent at first consideration. It would appear that the rate of evaporation could be measured by the rate at which water disappeared from an open pan exposed to the atmosphere. While such methods have been used they are subject to numerous errors and limitations. The rate of evaporation for such a pan is controlled not only by environmental conditions, but also by the size, shape, and color of the pan, and the depth of water below the rim. Other errors arise from the accumulation of rainwater and the splashing of water due to wind or other causes.

The many difficulties encountered in the use of open pans of water for measuring evaporation rates led to the devising of more suitable instruments for this purpose. Many measurements of evaporation rates have been made with instruments called *atmometers* (Fig. 38). These consist essentially of surfaces of porous clay moulded in the form of hollow cylinders or spheres. Water evaporates from such surfaces in essentially the same way that it evaporates from a free surface of water. Atmometers are attached to a reservoir of water as shown in the figure, and are usually provided with mercury valves which prevent absorption of rain. The loss of water from such instruments can be determined either by measuring the decrease in volume of water, or the loss in weight of the instrument. Although atmometers are not subject to many of the errors inherent in the use of open-pan evaporimeters numerous precautions must be observed in employing them. Details of atmometric technique are discussed by Livingston (1935).

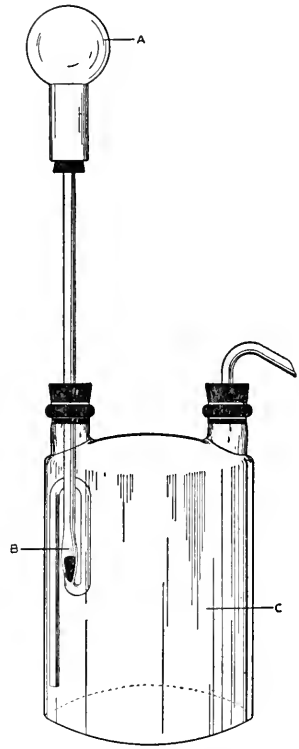

FIG. 38. Arrangement for the measurement of evaporation rates by means of an atmometer. (*A*) porous clay sphere, (*B*) mercury valve, (*C*) reservoir.

Measurement of Transpiration.—Four general methods are in use for measuring transpiration, but only the first two of these as listed can be used for quantitative determinations of the rate of loss of water-vapor from plants which are rooted in the soil.

1. *Method of Weighing Potted Plants.*—This method can be employed only with plants which are rooted in pots or other suitable containers. For

laboratory experiments potted plants are often used, the pot generally being enclosed in a metal shell, and the soil surface sealed off so that evaporational water loss can occur only through the plant. For field or large-scale experiments, metal receptacles have been found convenient in which case it is only necessary to seal off or cover the soil surface in such a way as to prevent evaporation. The method is limited in practical application to plants which can be grown in readily portable containers. The transpiration rates of plants as large as mature maize plants have been measured by this method.

The loss in weight of the container and plants for a given time interval may be considered to represent transpiration as the effect of other factors on the weight of the set-up is usually negligible. If the experiment is to be continued for more than a relatively short period it is necessary to provide the container with a watering tube through which known volumes of water can be introduced into and distributed throughout the enclosed soil at appropriate intervals.

In employing this method the receptacle in which the plants are growing may be weighed at selected intervals by manual manipulation or may be placed on a balance which is so arranged that each loss of a definite increment of weight (for example 1 g.) is automatically registered on a recording device (Transeau, 1911, Briggs and Shantz, 1915, Schratz, 1932, and others).

2. *Method of Collecting and Weighing Transpired Water-Vapor.*—This method requires a rather elaborate experimental set-up, but is the only way in which the rate of transpiration can be determined quantitatively for plants growing in out-of-door habitats. The plant is enclosed within a glass chamber, the soil surface sealed off, and a stream of air taken directly from the atmosphere circulated rapidly through this container. After passing through the chamber the air is conducted through tubes or vessels containing a water absorbent such as calcium chloride. The gain in weight of these absorption tubes during the experiment represents the water-vapor transpired by the plant plus the water-vapor which was introduced into the system from the outside atmosphere. In order to determine the proportion of the water-vapor which comes from the atmosphere it is necessary to set up a check apparatus in exactly the same manner, except that no plant is enclosed in the glass chamber, and to pass air through it at the same rate that it is circulated through the set-up containing the plant. The gain in weight of the water-absorption tubes in the second apparatus represents water-vapor from the atmosphere. Such a method has been used by Minckler (1936) for measuring transpiration from attached branches of trees.

3. *Potometer Methods.*—Of a limited usefulness for the measurement of transpirational water loss are instruments known as potometers, a type of

which is shown in Fig. 39. The severed stem of a plant is immersed in water in the reservoir of the potometer and the rate of water loss determined by the rate at which the volume of water in the apparatus shrinks. This is usually followed by noting the rate of movement of an air bubble in the water

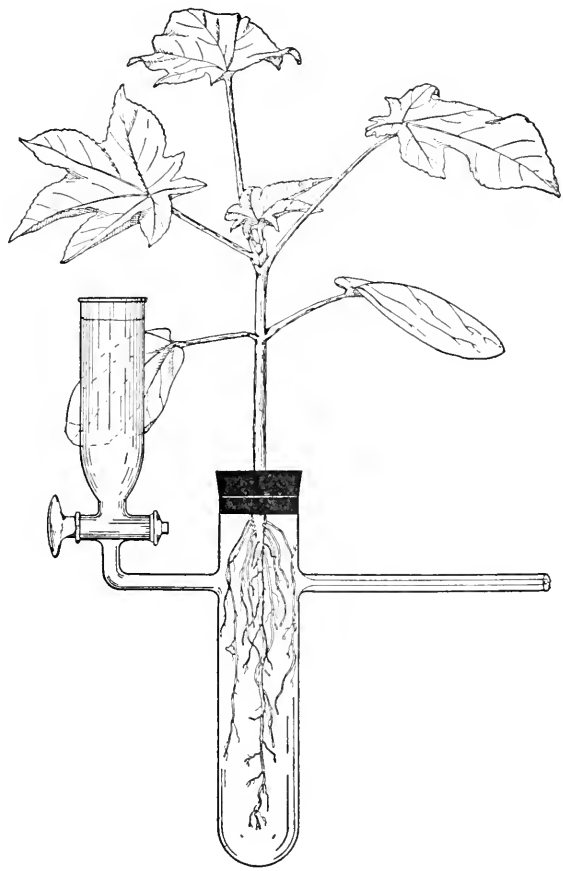

FIG. 39. Potometer as set up for measurement of the rate of absorption of water. The full length of the capillary tube is not shown.

in the capillary sidearm of the instrument. Some potometers, as shown in the figure, are so constructed that the entire root system of plants which have been specially grown for the purpose in solution cultures can be immersed in the reservoir of the instrument. A potometer actually measures the rate of absorption of water rather than the rate of transpiration. While under many

conditions the rates of these two processes are virtually equal, this is not always true, particularly if an internal water deficit exists in the plant. The rate of transpiration as measured for a cut shoot in a potometer does not necessarily bear any relation to its rate of transpiration while it was still attached to a plant. The reasons for this will become clear in the discussion of the internal water relations of plants (Chap. XVIII). The principal utility of potometers is in laboratory demonstrations of the effects of various environmental factors upon the rate of transpiration.

4. *Hygrometric Paper Methods.*—When filter paper is impregnated with a dilute (about 3 per cent) solution of cobalt chloride and dried it becomes a bright blue in color. If exposed to moist air its color gradually changes to pink. The same color change ensues when a piece of such paper is brought into contact with the transpiring surface of a leaf. If small pieces of such paper are so mounted as to be protected from the water-vapor of the atmosphere by glass, mica, or celluloid, and are brought into contact with the surface of a leaf the rate at which the paper changes in color from blue to pink is an indication of the rate at which water-vapor is being lost by that leaf. A leaf which changes the color of a piece of cobalt chloride from its full blue color to its full pink color in, for example, 30 seconds is losing water to the paper twice as fast as a leaf which requires 60 seconds to accomplish a similar change. This method gives no measure of absolute rates of transpiration, because when a portion of a leaf is covered with a piece of cobalt chloride paper the environmental conditions influencing the leaf under the paper are very different from those which would influence it were it freely exposed to the atmosphere. The leaf under the paper is exposed to a reduced light intensity and an initially lower vapor pressure than freely exposed parts of the same leaf, and furthermore, is completely isolated from any effects of wind. Hence the rate of loss of water to the paper may be very different from the rate of water loss of the same area of leaf surface to the atmosphere. Under certain conditions this method can be used for a determination of the *relative* rates of transpiration of different species with a fair degree of accuracy. Even for relative determinations of transpiration rates, however, this method gives valid results only when all of the plants are growing under essentially the same atmospheric conditions. Modifications and limitations of this method are discussed by Livingston and Shreve (1916) and Meyer (1927).

The transpiration rates of plants may be expressed in terms of a unit of leaf surface (usually per square decimeter), per unit of fresh or dry weight of the leaves or tops of the plant, or per plant. Each of these modes of expression of transpiration rates is of value for certain purposes.

The Magnitude of Transpiration.—The magnitude of transpiration, whether computed on the basis of a unit area of leaf surface, an entire plant, or an acre of forest or crop plants, shows a great variation depending both on the plant species and environmental conditions to which they are exposed. The quantity of water transpired may be computed and compared for hourly, daily, seasonal, or yearly periods. Because of the great fluctuations in transpiration rates with environmental conditions the citation of isolated values of transpiration rates is usually of very little physiological significance.

Transpiration rates in plants of temperate regions range up to about 5 g. per dm.^{2} of leaf surface per hour.[1] Usual rates, under favorable conditions for stomatal transpiration, will generally fall within a range of 0.5 to 2.5 g. per dm.^{2} per hour. At night, or during periods unfavorable to stomatal transpiration (dry soil conditions, etc.) the rate may fall to 0.1 g. per dm.^{2} per hour, or even less. Under favorable conditions many herbaceous plants will transpire several times their own volume of water in the course of a single day.

It is impossible to calculate accurately the transpiration rate of large plants such as trees from the known rate of transpiration of some of the leaves and an estimate of the area of all of the leaves on the tree. In the first place it is difficult to arrive at any accurate estimate of the aggregate leaf area of a large tree. In the second, there is a great variability in the transpiration rate among the different leaves. The rate of transpiration of the individual leaves varies depending upon their position, exposure, age, and internal physiological conditions. For example, Huber (1923), found that low twigs of a redwood tree transpired six times as rapidly as similar twigs from the height of 12 meters. Neither can it be assumed, as mentioned in the discussion of the potometer method, that the transpiration rate of leaves of branches which may have been removed from a tree and placed with their cut ends in water will be the same as their rate of transpiration before the branches were detached.

Hence attempts to determine the transpiration rate for an entire tree from values determined for a few of the leaves on that tree can only result in approximations. The same comments in general apply, although perhaps not so emphatically, to calculations of the total transpiration of fields of cultivated crops and natural vegetation areas based upon transpiration as determined for a limited number of individual plants. All such computations are

[1] If transpiration is measured for one minute intervals rates much in excess of this may be observed, but such rates are maintained only for relatively short periods of time.

of necessity approximations, although, not of course, without their utility in the consideration of certain types of problems.

The results of such calculations indicate that sufficient water may transpire from maize plants during the course of a season to cover the field in which they are growing to a depth of 15 inches (Transeau, 1926). Similarly Minckler (1936) has calculated that in a growing season water equivalent to 4.8 inches of rainfall may transpire from an acre of American elm trees and that an acre of red maples, growing in a very moist habitat, may lose water equivalent to 28.3 inches per acre. In evaluating all such data it must be remembered, however, that the magnitude of transpiration varies tremendously with the available soil water supply.

Significance of Transpiration.—Opinions regarding the significance of transpiration have ranged all the way from those which would put it practically on a par with such processes as photosynthesis and respiration, to those which would relegate it to the category of a "necessary evil" (Curtis, 1926). The principal rôles which have been ascribed to the transpiration of plants can be summarized under the following three headings:

1. *Supposed Rôle in the Movement of Water.*—It is often claimed or assumed that the movement of water through the plant requires the occurrence of transpiration from the leaves. That this concept is entirely erroneous will be clear from the discussion of the mechanism of the conduction of water through plants in Chap. XV. Under conditions of high transpiration the movement of water through plants is, in general, more rapid than under conditions of low transpiration. The mechanism causing ascent of water through a plant operates in such a way that any decrease in the turgidity of the mesophyll cells favors a more rapid movement of water towards those cells. Hence a rapid transpiration rate, which invariably results in a considerable loss of turgidity by the mesophyll cells, usually speeds up the rate at which water ascends through the plant. However translocation of water to the extent that it is used in photosynthesis, growth or other metabolic processes continues even if the transpiration rate is negligible.

2. *Supposed Rôle in the Absorption and Translocation of Mineral Salts.*—It has often been assumed that the more rapid the rate of transpiration, the greater the rate of absorption of mineral salts. This view implies that the dissolved mineral salts are swept into the plant along with the absorbed water, a postulation which ignores much evidence that the mechanisms operating in the absorption of water and the absorption of mineral salts are very different (Chap. XVII; Chap. XXIV).

The results of certain experiments do indicate that a somewhat larger quantity of mineral salts accumulates in plants under conditions favoring high

transpiration than in similar plants growing under conditions of low transpiration (Freeland, 1937) although there is no actual proportionality between the volume of water absorbed and the quantity of mineral salts which pass into the plant. Since even when plants grow under conditions favoring low transpiration rates they usually obtain an adequate quota of the various mineral salts, provided they are present in sufficient abundance in the soil, it is difficult to see that this effect is of any very great advantage to the plant. Realization is now fairly general that there is, at the most, only a slight correlation between the rate of transpiration and the rate of absorption of mineral salts from the soil.

It is also generally considered that transpiration plays an important part in the translocation of mineral salts from the root system to the top of the plant. That some upward translocation of mineral salts occurs in the transpiration stream is indisputable, but whether this is an important part of the total quantity transported is open to debate. There is considerable evidence that at least a part of the mineral salts which move through the plant are translocated in the phloem (Chap. XXVIII). The rate of transpiration can have little or no direct effect upon the movement of such salts. While high transpiration rates may mean a somewhat higher rate of transport of mineral salts through the plant than low ones it is doubtful if this is of great significance from the standpoint of the plant.

3. *Supposed Rôle in the Dissipation of Radiant Energy.*—Leaves exposed to direct sunlight absorb large quantities of radiant energy which, unless dissipated in some other way, will be converted into heat energy and raise the temperature of the leaf. The possibilities of such an effect are indicated by the following approximate calculations: In direct noon-day summer sunlight the rate of receipt of solar energy is often 1.3 g.-cal. per square centimeter of leaf surface per minute and not uncommonly even greater. The proportion of this actually absorbed varies with the kind of leaf, but will be assumed to be only 50 per cent, which is a conservative estimate. The incident radiant energy which is not absorbed by the leaf is all transmitted or reflected. Such a small proportion of the absorbed energy is used in photosynthesis that it can be neglected in a rough calculation. Hence about 0.65 g.-cal. of energy is absorbed per square centimeter of leaf surface per minute. If the mass of a square centimeter of a leaf is taken as 0.020 g. and its specific heat as 0.879 g.-cal.[2] the rise in temperature per minute would be $\frac{0.65}{0.020 \times 0.879}$ or about 37° C. Since the thermal death point of most plant protoplasm lies

[2] These are the actual values as determined for a sunflower leaf by Brown and Escombe (1905). See also Shull (1930).

between 50 and 60° C. it should not require more than two minutes to heat the leaves of most plants to a lethal temperature. Actual measurements of leaf temperatures, however, show that they seldom even approach their thermal death points. Leaf temperatures usually do not exceed atmospheric temperatures by more than a few degrees centigrade. Evidently some efficient energy-dissipating mechanism is at work which prevents the accumulation of heat energy in leaves.

Since transpiration is an energy-consuming process it has often been assumed that it is in the evaporation of water from the leaves that most of the energy absorbed by them is dissipated. We might well inquire, therefore, regarding the possible efficiency of transpiration as an energy-dissipating process. The evaporation of a gram of water at 20° C. requires 584 g.-cal. For the dissipation of 0.65 g.-cal. of heat, therefore, the evaporation of 0.0011 g. $\left(\frac{0.65}{584}\right)$ of water per square centimeter of leaf surface per minute is required. This is equivalent to 6.6 g. ($0.0011 \times 100 \times 60$) of water per square decimeter of leaf surface per hour, a rate of transpiration which is seldom if ever attained by plants for any sustained period of time. Evidently transpiration, even when occurring at its maximum rate, can at the most account for a dissipation of only part of the radiant energy which is absorbed by leaves in direct sunlight.

The fact that transpiration is often inadequate in direct sunlight to account for the dissipation of all the absorbed radiant energy leads naturally to the question of whether it is at all essential for this process. Observations have shown that leaves in which the transpiration rate is greatly reduced, as for example those in which the stomates are plugged with vaseline, leaves in a wilted condition, or the leaves of plants in xeric habitats during dry seasons, in which the occurrence of any appreciable amount of transpiration is precluded by the lack of soil water, seldom have temperatures which are anywhere near the thermal death point, even when exposed to direct sunlight. Although transpiration often accounts for the dissipation of some or even most of the absorbed radiation, in so doing it apparently plays no essential rôle since absorbed radiation can be dissipated by purely physical means. As soon as the temperature of a leaf exceeds that of the surrounding atmosphere, it will lose heat to the atmosphere in the same way that any other object heated above its environmental temperature does, *i.e.* by one or more of the purely physical processes of conduction, radiation, and convection. The term *thermal emissivity* is frequently applied to this physical loss of heat energy by leaves and other objects. As will be shown in Chap. XIV, the thermal emissivity

of leaves is adequate to account for the dissipation of all absorbed radiant energy.

Actually, instead of benefiting plants, transpiration may often be detrimental. Under conditions of deficient soil water or of high transpiration rates even when the soil water supply is adequate, this process results in a diminution in the water content of a plant and in the turgidity of its cells. Prolonged drought conditions ultimately result in a severe desiccation with the consequent death of all except the most drought resistant species. When the diminution in water content is less severe, a train of other effects such as a decrease in the turgidity of the cells, stomatal closure and reduction in rate or cessation of photosynthesis are induced, all of which have the end result of checking the growth of the plant. It is probably true that lack of water is more often the limiting factor in plant growth than any other single factor. Furthermore deficiency of water is probably responsible for the death of more plants under natural or even cultural conditions than any other single cause.

The fundamental effects of transpiration upon the plant are not to be sought in any hypothetical "advantages" of the process to the plant, but in its very real and readily ascertainable influences upon the water relations of plant cells and tissues, and through these its effects upon other plant processes. In spite of the fact that transpiration may be regarded in a sense as an incidental phenomenon, its indirect influence upon the metabolic processes of plants is a profound one. It is this fact, more than any other, which justifies intensive and critical studies of this process.

The Loss of Water from Plants in Liquid Form.—If a pot of young oat plants be copiously watered and then enclosed in a bell jar, in a relatively short time a slow exudation of water begins at the tip of each leaf. The drops which form at the leaf apices gradually enlarge and eventually may run down the side of the leaf or fall off. This process of the escape of liquid water from uninjured plants is called *guttation* (Fig. 40). It is of very general occurrence in plants, having been recorded in plants of more than 300 genera although there are many species in which it has not been observed. Guttation occurs most frequently and abundantly under conditions which favor rapid absorption of water by the roots, but which result in a reduced rate of transpiration. In most temperate regions such conditions occur most frequently during the late spring when there is often an alternation between relatively cool nights and relatively warm days. Guttation is frequently observed at that season, usually taking place at night or during the early hours of the morning. The drops of guttation water which form at the tips

of grass blades and the tips or edges of the leaves of other plants are often erroneously considered to be dew.

The process of guttation occurs from specialized structures known as *hydathodes* (Fig. 41). These structures are also sometimes referred to as *water stomates* or *water pores*. As the figure indicates a typical hydathode consists of an enlarged stomate-like opening below which is a rather large

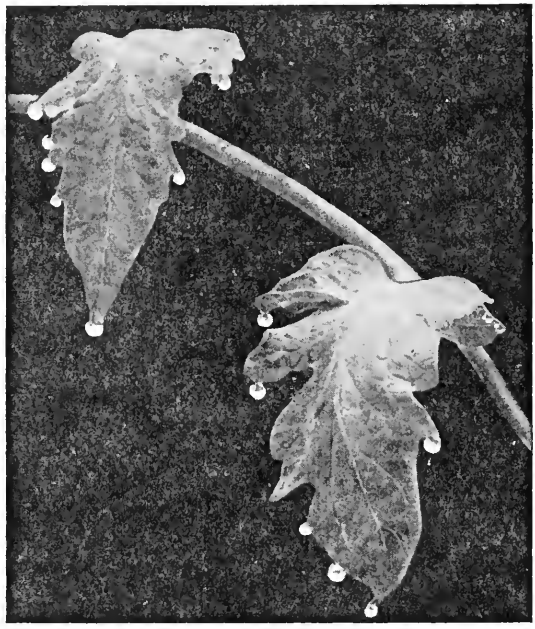

FIG. 40. Guttation from tomato leaves. Photograph, courtesy of Dr. J. H. Gourley.

chamber, bordered by a mass of thin-walled, loosely arranged cells called the *epithem*. The xylem elements of a vascular bundle terminate just below the epithem.

The exudation of water through hydathodes is considered to result from a pressure which develops in the sap of the xylem elements, and not to any locally developed pressure in the hydathode itself. This pressure is generally believed to be identical with the so-called "root pressure" (Chap. XV). It is supposed that the water is forced from the vessels through the intercellular spaces of the epithem layer and out of the plant through the pore of the hydathode. This water is not pure but contains traces of sugars and other solutes. While the volume of water exuded by most temperate region plants in guttation is usually insignificant, some tropical species lose large quantities in this process. A young leaf of *Colacasia nymphaefolia*, a

native of India, has been observed to lose as much as 100 cc. of liquid water in a single night by guttation.

Glands are found on leaves, flower parts and other organs of the plant. Many of them represent modified epidermal hairs or other epidermal cells. Certain types of glands secrete water or, more accurately, a dilute sap. These have sometimes been classed as hydathodes, but it seems better to restrict this term to the type of structure previously described under this name. The exudation of water by glands is generally called *secretion* and is apparently caused by forces which develop within the gland itself, and not by a pressure developed in the sap of the water conducting tissue as appears to be true of hydathodes. Glands which secrete water or dilute saps are not closely connected with xylem elements as are the hydathodes. The mechanism of this process is unknown. Many glands are found in plants which secrete other substances as well as water. Among these are sugars (as in nectar), calcium salts, resins, volatile oils, and enzymes.

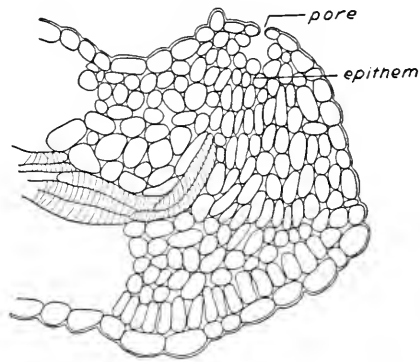

FIG. 41. Hydathode at the margin of a tomato leaf as seen in sectional view (semi-diagrammatic). Note termination of vessels just back of the epithem.

If the stem of an herbaceous plant be cut or broken a slow exudation of sap often occurs from the ruptured stump. A similar phenomenon can often be observed in the cut or broken stems or branches of woody plants, especially in the spring of the year. The exudation of sap from holes bored into maple trees is a good example. This phenomenon has long been called *bleeding*. Under certain conditions large quantities of dilute sap may be lost in this process. A single vigorous grape vine may lose as much as one liter of sap in a day, while under favorable conditions a sugar maple tree will yield from five to six liters in twenty-four hours.

In some species bleeding appears to be due to a "root pressure" developed in the xylem elements, in others to pressures developed in the phloem, and in still others to locally developed pressures in other tissues. The significance of the various internal pressures which develop in the conductive tissues of plants will be discussed later, particularly in relation to the problems of the translocation of water and solutes in plants (Chap. XV; Chap. XXVIII).

Discussion Questions

1. How could you demonstrate experimentally that a much larger quantity of water is lost by stomatal transpiration in most species than by cuticular transpiration?
2. Is it harmful to prune grapevines or other woody plants when they will bleed profusely?
3. Explain how a mercury valve (Fig. 38) works in preventing entrance of rain into an atmometer reservoir.
4. When a leaf in bright light is surrounded by a bag of cellophane, which checks transpiration, the temperature of the leaf rises rapidly. This has been interpreted as indicating that rapid heating of a leaf would occur if transpiration ceases. What is a more probable interpretation of this effect?
5. When computing transpiration per unit area of leaf surface should both upper and lower epidermis be considered as evaporating surfaces or only one?
6. What method or methods of measuring transpiration would you recommend for determining: (a) the transpiration rate of single leaves from various heights on the same tall oak tree? (b) the relative and absolute amounts of stomatal and cuticular transpiration of a potted plant? (c) the effect of high and low humidity upon the transpiration of potted geranium plants? (d) the transpiration of small plants growing in a solution culture? (e) the effect of oil sprays on the transpiration of peach trees? Explain reasons for your choices.

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CHAPTER XIII

THE STOMATAL MECHANISM

The most important physiological fact about the stomates is that they are sometimes open and sometimes closed. When open they serve as the principal pathways through which gaseous exchanges take place between the intercellular spaces of the leaf and the surrounding atmosphere. When closed all gaseous exchanges between a leaf and its environment are greatly retarded. The gases of greatest physiological importance which enter or depart from a leaf principally through the stomates are oxygen, carbon dioxide and water-vapor. The movement of gases through the stomates in either direction is primarily a diffusion phenomenon, although under certain conditions, as for example when the intercellular spaces are alternately compressed and expanded by bending of the leaf in a high wind, alternate outward and inward mass movements of gases may occur. Although the stomates are the principal portals through which entry and escape of gases takes place, the fact should not be overlooked that at least small amounts of these three gases pass directly through the epidermis and cuticular layers of all leaves. In submerged aquatics all gaseous exchanges between the plant and its environment occur through the epidermis.

Structure of the Stomates.—The *stomates* or *stomata* (singular *stomate* or *stoma*) are minute pores which occur in the epidermis of plants. They are surrounded by two specialized epidermal cells known as the *guard cells.* Stomates may occur on any part of a plant except the roots, but in most species are most abundant upon the leaves. The size of the stomatal pore varies in most plants depending upon the turgidity of the guard cells and often, especially at night, it is entirely closed. In Fig. 42 are depicted surface and cross-sectional views of several of the commoner types of stomates. The structure of stomatal apparatus shows marked variations in detail in different species of plants, but the essential feature of a pore between two modified epidermal cells is common to all species of vascular plants. Guard cells which are roughly kidney or bean-shaped as seen in surface view are typical of more species of plants than any other kind (Fig. 42, *A*). In some species the epidermal cells bordering on the guard cells are different in con-

figuration from other cells in the same tissue; these are called *subsidiary cells* or *accessory cells* (Fig. 42, *B*). In many species of the grass and sedge families the guard cells are distinctly elongate (Fig. 42, *C*). In most species of conifers, and in certain other species, stomates are of the "sunken" type

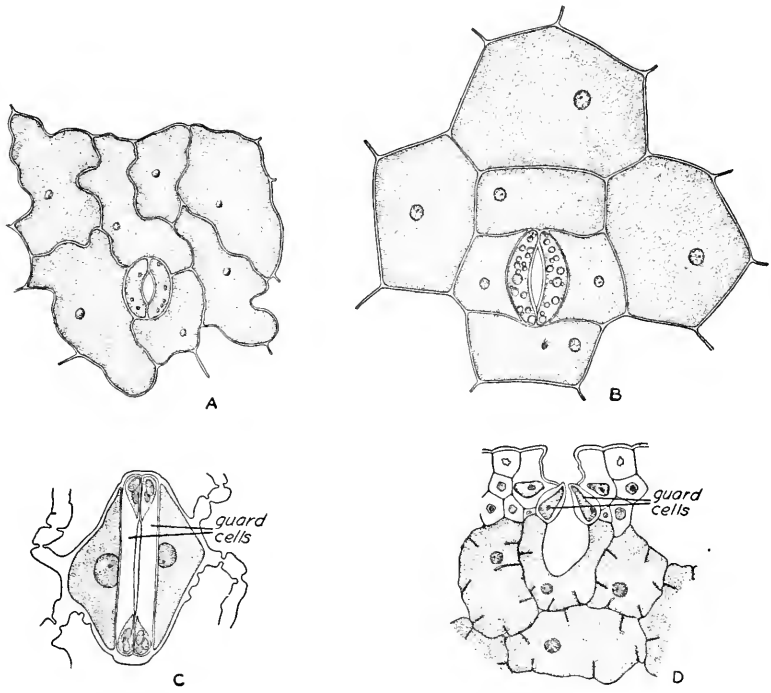

FIG. 42. Structure of stomates. (*A*) surface view of sunflower stomate, (*B*) surface view of *Zebrina* stomate, showing four subsidiary cells around guard cells, (*C*) surface view of maize stomate, (*D*) cross sectional view of Austrian pine stomate.

(Fig. 42, *D*). A perspective view of a stomate and the surrounding guard cells is shown in Fig. 43.

The guard cells differ from the other epidermal cells in that they contain plastids which are green in color. These have often been assumed to be chloroplasts, and are frequently so-called, but the green pigment present does not seem to be true chlorophyll as photosynthesis apparently does not occur in the guard cells.

Size and Distribution of Stomates.—The size of the stomatal pore varies greatly according to the species of plant, and somewhat even among

the individual stomates on any one plant. They are always very minute, however, their dimensions being expressed in terms of microns. Measurements of the average length and breadth of the fully open stomatal pore on a number of representative species of plants are listed in Table 21.

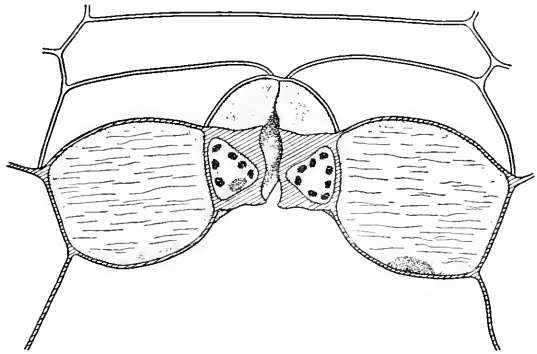

FIG. 43. Perspective view of a stomate and adjacent cells (semi-diagrammatic).

Minute as these openings appear to be from a human scale of values, they are enormous when compared with the size of the gas molecules which diffuse through them. The calculated diameter of a water molecule is 0.000454 μ. More than 2,000 water molecules would have to be placed side by side to measure a distance of 1 μ. The molecules of both carbon dioxide and oxygen are larger than water molecules. Since the stomatal diameters usually are considerably in excess of 1 μ, it is evident that the stomates afford relatively enormous portals to the gas molecules which diffuse through them.

In general the number of stomates present in the epidermis of leaves may range from a few thousand to over a hundred thousand per square centimeter, the exact number depending upon the species and upon the environmental conditions under which the leaf has developed. A single maize plant has been estimated to bear from 140 to 240 million stomates, while the number on a large tree could be expressed only by a figure of astronomical dimensions.

The average numbers of stomates which have been found per square centimeter on the leaves of a number of representative species are listed in Table 21. However, marked deviations from such average values are possible for any species, depending upon the environmental conditions under which the leaves have developed. The number of stomates per unit area of leaf surface may be quite different on leaves of two plants of the same species, if one grew in a greenhouse, and the other grew in the open, or upon the

TABLE 21—SIZE AND DISTRIBUTION OF STOMATES ON THE LEAVES OF VARIOUS SPECIES OF PLANTS (DATA OF ECKERSON, 1908; SALISBURY, 1927; KISSER, 1929; MILLER, 1931; AND YOCUM, 1935)

Species	Ave. no. of stomates per cm²		Size (length × breadth) of pore when fully open (lower epidermis)	Reference
	Upper epidermis	Lower epidermis		
Alfalfa (*Medicago sativa*)	16,900	13,800	.....	M
Apple (*Pyrus malus* var.)	0	29,400	.....	M
Barberry (*Berberis vulgaris*)	0	22,900	.....	K
Bean (*Phaseolus vulgaris*)	4,000	28,100	7 × 3 μ	E
Begonia (*Begonia coccinea*)	0	4,000	21 × 8 μ	E
Black Oak (*Quercus velutina*)	0	58,000	.....	Y
Black Poplar (*Populus nigra*)	2,000	11,500	.....	S
Black Walnut (*Juglans nigra*)	0	46,100	.....	K
Cabbage (*Brassica oleracea*)	14,100	22,600	.....	M
Castor Bean (*Ricinus communis*)	6,400	17,600	10 × 4 μ	E
Cherry (*Prunus cerasus* var.)	0	24,900	.....	M
Coleus (*Coleus blumei*)	0	14,100	10 × 5 μ	E
English Ivy (*Hedera helix*)	0	15,800	11 × 4 μ	E
English Oak (*Quercus robur*)	0	45,000	.....	S
Geranium (*Pelargonium domesticum*)	1,900	5,900	19 × 12 μ	E
Holly (*Ilex opaca*)	0	17,000	12.5 × 6.5 μ	K
Jimson Weed (*Datura stramonium*)	11,400	18,900	.....	K
Lilac (*Syringa vulgaris*)	0	33,000	.....	K
Linden (*Tilia vulgaris*)	0	13,000	.....	S
Maize (*Zea mais*)	5,200	6,800	19 × 5 μ	E
Mulberry (*Morus alba*)	0	48,000	.....	K
Nasturtium (*Tropaeolum majus*)	0	13,000	12 × 6 μ	E
Nightshade (*Solanum dulcamara*)	6,000	26,300	.....	K
Oat (*Avena sativa*)	2,500	2,300	38 × 8 μ	E
Pea (*Pisum sativum*)	10,100	21,600	.....	K
Peach (*Prunus persica* var.)	0	22,500	.....	M
Potato (*Solanum tuberosum*)	5,100	16,100	.....	M
Red Oak (*Quercus rubra*)	0	68,000	.....	Y
Scarlet Oak (*Quercus coccinea*)	0	103,800	.....	Y
Scilla (*Scilla nutans*)	5,500	5,100	.....	S
Sunflower (*Helianthus annuus*)	8,500	15,600	22 × 8 μ	E
Sycamore (*Platanus occidentalis*)	0	27,800	.....	K
Tomato (*Lycopersicon esculentum*)	1,200	13,000	13 × 6 μ	E
Tree of Heaven (*Ailanthus glandulosa*)	0	38,600	.....	K
Wandering Jew (*Zebrina pendula*)	0	1,400	31 × 12 μ	E
Wheat (*Triticum sativum*)	3,300	1,400	38 × 7 μ	E
Willow Oak (*Quercus phellos*)	0	72,300	.....	Y
Wood Sorrel (*Oxalis acetosella*)	0	4,500	.....	S
Yew (*Taxus baccata*)	0	11,500	.....	S

leaves of plants of the same species which have developed during different seasons.

As shown in Table 21, stomates occur in both the upper and lower epidermis of many species of plants. In numerous others, especially woody species, they are confined to the lower epidermis. Even in those species in which stomates occur on both surfaces of the leaf they are commonly, but not always, more abundant in the lower epidermis. In floating leaves such as those of the water-lily, stomates occur only in the upper epidermis. Species in which the stomates are relatively small usually have more per unit area than species in which the stomates are relatively large.

The number of stomates per unit area usually varies on different leaves of the same plant, and even in different parts of the same leaf. On individual leaves it appears to be generally true that the greatest number of stomates per unit area is at the tip, the lowest towards the base, while the middle portion of a leaf shows a frequency midway between these two extremes. As a rule, the higher the point of attachment of a leaf on the stem of a plant, the greater the number of stomates per unit area.

In general no correlation has been found between transpiration rates and either the size or distribution of stomates, other factors being much more important in determining the rate of loss of water-vapor from the intercellular spaces.

Principles Governing Diffusion of Gases through the Stomates.—Since virtually all gaseous exchanges between the intercellular spaces and the atmosphere take place through the stomates, the problem of the *diffusive capacity* of the stomatal pores is an important one. Although the aggregate area of the fully open stomates on a single leaf is seldom more than 3 per cent of the leaf area and is often as low as 1 per cent, the rate of water loss from the leaves (*i.e.* loss per unit area in a unit time) in many species may be, under favorable conditions, as much as 50 per cent of the evaporation from an exposed water surface of comparable dimensions. Stålfelt (1932) records that transpiration from a leaf of birch (*Betula pubescens*) under the most favorable conditions may be 65 per cent of the evaporation from a comparable evaporating surface, but this is probably an unusually high value. It is therefore evident that water-vapor often diffuses through the stomates at rates ranging up to at least 50 times greater than it diffuses away from an equal area of exposed evaporating surface.

This unexpectedly high diffusive capacity of the stomates is intelligible in terms of the results of studies upon the principles of diffusion through small apertures. The classical investigation of this problem was made by Brown and Escombe (1900) who studied the rate of diffusion of carbon

dioxide through tubes of known dimensions (Chap. XXI). They first made the important discovery that if a septum which had been pierced with a small circular aperture was interposed across a column of diffusing gas, that the rate at which carbon dioxide diffused through this aperture was much greater than the rate at which it passed through an equal area of the unobstructed tube.

Since the problem at present under consideration is the diffusion of water-vapor rather than carbon dioxide through small apertures, the data presented in Table 22 will be used to illustrate more fully the principles regarding the diffusion of gases through small openings. These data were obtained by sealing thin septa, through the center of which were cut round openings of known dimensions, across the circular mouths of small bottles which had previously been filled to a certain level with water, and then determining the loss in weight of each bottle after all of them had been kept under uniform conditions for the same period of time.

TABLE 22—DIFFUSION OF WATER-VAPOR THROUGH SMALL OPENINGS UNDER UNIFORM CONDITIONS (DATA OF SAYRE, 1926)

Septum	Diameter of pores, mm.	Loss of water-vapor, grams	Relative areas of pores	Relative perimeters (circumferences) of pores	Relative amounts of water-vapor lost
1	2.64	2.655	1.00	1.00	1.00
2	1.60	1.583	0.37	0.61	0.59
3	0.95	0.928	0.13	0.36	0.35
4	0.81	0.762	0.09	0.31	0.29
5	0.72	0.672	0.07	0.27	0.25
6	0.65	0.590	0.06	0.25	0.22
7	0.56	0.492	0.05	0.21	0.18
8	0.48	0.455	0.03	0.18	0.17
9	0.41	0.393	0.02	0.15	0.15
10	0.35	0.364	0.01	0.13	0.14

The results of this experiment illustrate two important general principles: (1) The quantities of water-vapor diffusing through small openings in a given period of time are proportional (essentially) to the perimeters (circumferences) and not to the areas of the pores. This is shown by the close correspondence of the figures in the last two columns of Table 22. (2) The smaller the pore, the greater the water loss per unit area. The pore in septum 2, for example, has an exposed area only slightly more than one-third as great as the pore in septum 1, but diffusion through it is nearly two-thirds

as great as through septum 1. Similarly septum 10, with only half the exposed area of septum 9, permits almost as much diffusion to occur as through septum 9. These results indicate that the rate of diffusion of water-vapor through a small aperture is greater than through an equal area of a larger evaporating surface.

In other experiments, Sayre found that diffusion of water-vapor through small openings of elliptical shape is also more nearly proportional to their perimeters than to their areas. Hence the "perimeter law" for the diffusion of gases also holds for openings of other than circular shape. An important inference which can be drawn from this finding is that a stomate attains almost its maximum diffusive capacity long before it is fully open, since the perimeter of a stomatal pore does not increase greatly after the aperture between the guard cells has widened perceptibly.

The fact that the diffusion of gases through small apertures is much more nearly proportional to their perimeters than to their areas is due to the more rapid diffusion of the molecules through an opening at the periphery than through its center. A molecule of the diffusing gas at the rim of the hole is surrounded on all sides by other molecules moving in all directions. The concentration of diffusing molecules is much less in an outward direction from the rim, however, than towards the center of the opening. Furthermore, the diffusion gradient is much steeper from the rim outwards than at points above the opening. Hence the number of molecules escaping through the hole from a given point near the rim per unit time interval will greatly exceed the number escaping from a point near the center of the opening.

Reduction in the area of any circular or elliptical opening results in increasing its perimeter relative to its area. Hence in small apertures such a large proportion of the diffusion occurs at the edge of the opening that the effect of any diffusion which occurs through its center is almost if not completely obscured, and measurements show the rate of diffusion through the small apertures to be essentially proportional to their perimeters. In fact in an opening 0.5 cm. in diameter, for example, a large part of the center of the aperture can be entirely blocked off without any very great effect upon the rate of diffusion of a gas through it.

That the diffusion of gases through small apertures occurs largely at the rim of the hole can be demonstrated visually by means of a simple experiment. A cylindrical glass vessel is partly filled with a gelatin sol which is allowed to gel. A small opening is punched through the center of a disk of celluloid which is then inserted into the position on top of the gelatin, the gap between the edge of the disk and the wall of the cylinder being

sealed with melted vaseline in order to prevent leaks. A solution of potassium permanganate, which is purple in color, is now introduced into the vessel on top of the disk (Fig. 44). After a few hours the molecules of potassium permanganate, after diffusing through the aperture, become distributed in such a way as to occupy a hemispherical zone below the opening. The hemispherical distribution assumed by molecules which are diffusing through a small opening is often spoken of as a *diffusion shell.* Gases also form diffusion shells above small pores, but their configuration is often relatively unstable due to their ready distortion by wind or convection currents.

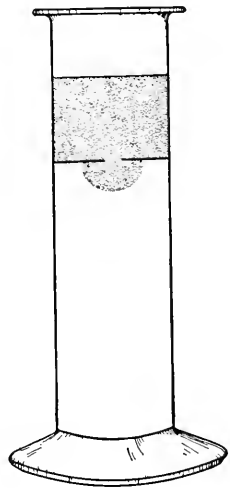

FIG. 44. Formation of a diffusion shell of potassium permanganate upon diffusion into gelatin.

Actually when molecules are diffusing through a small orifice there is formed not one, but two diffusion shells, one on each side of the diaphragm which is pierced with the opening. The experiment described above permits discernment of the hemispherical pattern of diffusion only on the receiving side of the system. There is also a second shell, a mirror image of the first, adjacent to the aperture on the side of the system from which diffusion is occurring. The deep color of the permanganate solution prevents visible detection of this second shell in the experiment described above.

Diffusion of gases from or into a leaf through the stomates involves a much more complex system than is represented by a septum pierced by a single aperture. Diffusion is occurring, not through a single opening, but simultaneously through thousands of minute apertures which are relatively close together. Experiments have been performed in analogous physical systems in which multiperforate instead of uniperforate diaphragms have been used. The results of experiments on different spacings of the pores in a septum upon the rate of diffusion of water-vapor per pore and per septum are depicted graphically in Fig. 45 and Fig. 46 respectively.

As shown in Fig. 45, the diffusion *per pore* increases with increase in the distance apart of the apertures, although not proportionately. With pores of this diameter (0.3 mm.) nearly the maximum diffusive capacity is attained when they are spaced at intervals of 20 diameters. The closer together the pores in a septum the greater the overlapping of the diffusion shells. The molecules diffusing through each aperture invade in part the zones into which the molecules passing through neighboring pores would diffuse were each opening the only one in the septum. Hence the diffusion gradients are less

steep than they would be were there only one pore in the septum. As a result the rate of diffusion through each pore is less than it would be through a pore of equal dimensions in a uniperforate septum.

The loss of water-vapor by diffusion *per septum* decreases with increase in the distance between the openings, *i.e.* with decrease in the number of pores per septum (Fig. 46). The decrease in diffusive capacity is not, however, in proportion to the reduction in the aggregate area of the pores in the septum. For example, when the pores are spaced 5 diameters apart their aggregate area is only 3.38 per cent of the septum area, yet diffusion through

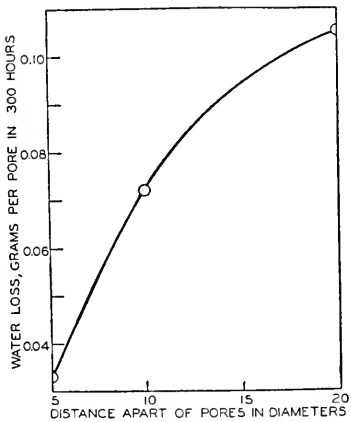

FIG. 45. Relation between water loss per pore and distance in diameters between pores. Data of Weishaupt (1935).

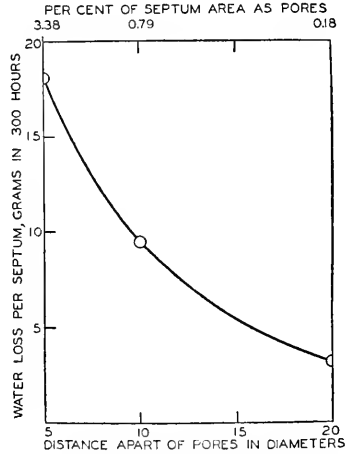

FIG. 46. Relation between water loss per septum and distance in diameters between pores. Data of Weishaupt (1935).

them was 62 per cent of that through an open bottle with a mouth of the same area as the septum (see Table 33).

The dimensions of the pores (0.3 mm. diameter, 0.0707 mm.^{2} area) used in the experiments just discussed are much greater than those of the average stomate. What about the diffusive capacity of still smaller pores? Huber (1930) has shown that the smaller and more numerous the pores, *aggregate pore area being constant*, the greater the diffusion per septum.[1] For example, when pores 0.05 mm. in diameter were used, occupying 3.2 per cent of the

[1] If equal-sized pores are spaced equidistantly, and represent the same proportionate area of the septum, they will be the same distance apart *in terms of their diameters*, regardless of pore size.

septum area, diffusion was 72 per cent as great as from an open evaporating surface (*cf.* with the value given above for pores 0.3 mm. in diameter).

Huber's results indicate that the smaller the pores for a given aggregate pore area the more nearly the diffusion through the septum will approach that of a free evaporating surface. In the sunflower and undoubtedly also in some other species the aggregate area of the stomatal pores is about 3 per cent of the leaf area. If diffusion through pores 0.05 mm. in diameter, occupying 3.2 per cent of the area of the septum, is 72 per cent of that from a free evaporating surface, diffusion through the much smaller stomates of this species (Table 21) should be proportionately greater. In such species it seems probable that the aggregate diffusive capacity of the stomates may closely approach that of a free evaporating surface. Even in species in which the total area of the stomatal pores is less than in the sunflower their aggregate diffusive capacity is very large relative to their area. The relatively high rates of water loss which occur from leaves in proportion to the area of the stomatal pores are thus explicable in terms of the principles of diffusion through multiperforate septa.

The theoretical diffusive capacity of the stomates is more than adequate to account for known rates of transpiration. This is shown by the calculations of Brown and Escombe (1900) for sunflower leaves. Assuming a leaf temperature of 20° C., the intercellular spaces to be saturated with water-vapor at that temperature (vapor pressure = 17.54 mm. Hg), and the vapor pressure of the atmosphere to be one-fourth of this value, their computations show that transpiration would occur at the rate of 17 g. of water-vapor per square decimeter of leaf surface per hour. This is several times greater than the maximum rate of transpiration ever recorded for a sunflower plant. Evidently some other factor than the diffusive capacity of the stomates has a limiting effect upon transpiration whenever the stomates are widely open.

The foregoing discussion of the diffusion of gases through small openings has been based on the assumption that diffusion is occurring into a quiet atmosphere. If an air current is blowing across the surface of a multiperforate septum with a sufficient velocity to prevent the formation of diffusion shells, the result is a steeper diffusion gradient and hence a greater rate of diffusion of gas through the pores of the septum. Hence, when subjected to air movements, the diffusion of water through multiperforate septa or the stomates may be even greater than the preceding discussion has indicated. Within limits increase in the velocity of the wind results in a progressive increase in the rate of diffusion through a multiperforate septum (Deneke, 1931).

The Mechanism of the Opening and Closing of the Stomates.—The degree of stomatal opening is influenced both by changes in the turgor of the

guard cells and by changes in the turgor of the epidermal cells, although the former usually play a predominant role. In general an increase in the turgor of the guard cells relative to that of the epidermal cells leads to a widening of the stomatal aperture, and *vice versa*. The greater this turgor difference, the wider the stomatal aperture.

The mechanism of the effect of changes in the turgor of the guard cells upon the size of the stomatal aperture varies with the structure, form, and position of the stomates. In one type of guard cell, found in many different species of plants, the cell wall is thicker on the side bordering the stomatal pore than on the side bordering the epidermal cells (Fig. 43). With an increase in turgor the thinner walls of the guard cells are stretched more than the thicker; this causes the thicker-walled sides to assume a concave shape, and results in the appearance of a gap—the stomatal pore—between the two guard cells. Opening of the stomates typical of the grass and sedge families (Fig. 42, *C*), appears to be due to swelling of the ends of the guard cells thus separating the abutting walls of the middle portion of the two adjacent guard cells. In the sunken stomates typical of conifers (Fig. 42, *D*), opening of the stomates seems to result largely from a change in the shape of the guard cells due to an increased turgor which is unaccompanied by any appreciable stretching of the walls. These various types of stomatal mechanisms are discussed by Copeland (1902).

The three principal factors which influence the opening and closing of the stomates are: (1) light, (2) the internal water relations of the leaf, and (3) temperature.

1. *Influence of the Light Factor in Stomatal Opening and Closing.*— The most familiar of all stomatal reactions is their response to light. Unless other conditions, to be discussed later, are limiting, the stomates of most species open when exposed to light, and close upon the failure of illumination. Most commonly, therefore, the stomates are open in the daytime and closed at night, although there are many exceptions to this statement. The sensitivity of stomates to the light factor probably varies considerably according to species.

Within limits the stomates appear to respond quantitatively to the amount of light absorbed. Stomatal opening apparently will occur in all wavelengths of the visible spectrum, although the influence of radiations in the red region appears to be weaker than the influence of other wavelengths of the visible spectrum (Sierp, 1933).

Upon the cessation of illumination the stomates usually begin to close. Generally this is a gradual process and, according to Stålfelt (1929) the greater the quantity of light which has been absorbed during the course of a day, the longer it takes for the completion of stomatal closure.

The work of Sayre (1926), Scarth (1932) and others indicates that the mechanism whereby light brings about stomatal opening and the mechanism whereby its absence causes stomatal closure are primarily osmotic, but are conditioned by changes in the H-ion concentration of the guard cells. Illumination of the guard cells has been found to result in a decrease in their H-ion concentration; failure of illumination in an increase. Thus Scarth found the pH of the guard cells of the Wandering Jew (*Zebrina pendula*) to range from 5.0 or less in the dark to between 6.0 and 7.4 in the light. The other cells of the leaf do not change appreciably in H-ion concentration upon the advent or failure of illumination, suggesting that the guard cells are less effectively buffered than the other leaf cells. Although several explanations have been offered to account for the effects of light and darkness on the pH of the guard cells none of them are strongly supported by experimental evidence and they will not be discussed.

The guard cells apparently always contain starch, but the quantity present is not constant from one hour of the day to the next. Sayre (1926) has shown that the starch content of the guard cells is at its maximum during the night, decreases rapidly during the daylight hours, and increases again towards evening. The sugar content of the guard cells bears a reciprocal relationship to the starch content; when the latter is high, the sugar content is low, and *vice versa*. These reciprocal changes are apparently the result of reversible reactions in which the total amount of carbohydrate involved does not vary greatly. Conversion of starch to sugar and of sugar to starch results from the action of the complex of enzymes known as "diastase" (Chap. XXVII). Increase in the pH of the guard cells such as occurs upon the incidence of light, appears to favor the hydrolytic (starch to sugar) action of diastase. On the contrary a decrease in their pH, such as occurs in the evening, favors the synthetic action of this enzyme whereby sugar is converted into starch.

Increase in the sugar concentration of the guard cells results in an increase in their osmotic pressure while a decrease in their sugar concentration has the opposite effect. That such changes in the osmotic pressure of the guard cells actually occur has been shown by many investigations. The diurnal changes in the osmotic pressures at incipient plasmolysis of the guard cells and epidermal cells of the English Ivy (*Hedera helix*) are shown in Fig. 47. In general the osmotic pressure of the guard cells is usually relatively high during the daylight hours, and relatively low at night. The osmotic pressure of the epidermal cells does not change appreciably during the course of the day and approximates that of the guard cells at night.

Increase in the osmotic pressure of the guard cells in the morning results

in an increase in their diffusion pressure deficit relative to that of the contiguous cells. Water therefore moves into the guard cells, increasing their turgor, which in turn leads to a widening of the stomatal aperture. Similarly a decrease in the osmotic pressure of the guard cells results in a diminution of their turgor and a narrowing of the stomatal aperture.

However, certain facts suggest that the effects of light upon stomates cannot be interpreted entirely in terms of the osmotic mechanism just described. One of these is the rapidity with which stomatal opening occurs.

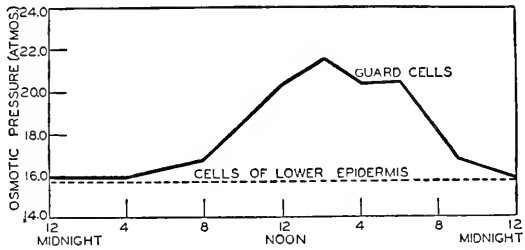

FIG. 47. Daily variations in the osmotic pressure of the guard cells and epidermal cells of English ivy (*Hedera helix*). Data of Beck (1931).

In some species at least the stomates open within less than a minute after exposure to light. It is difficult to visualize such a rapid action in terms of an enzymatic reaction, since usually such reactions occur at a relatively slow rate. Some investigators consider therefore that light exerts a direct effect upon the guard cells in addition to its indirect influence upon the magnitude of their osmotic pressure. Little if anything is known, however, concerning the mechanism of any such reaction.

2. *Influence of the Water Factor in Stomatal Opening and Closing.*— As subsequent discussion will show (Chap. XVIII) development of an internal water deficit in plants is of frequent occurrence, especially on warm summer days. A shrinkage in the total volume of water in a plant results in general in a diminution in the volume of water in each individual cell, although all cells will not necessarily be affected equally. Such a decrease in the water content of the leaf cells, not sufficient to induce visible wilting, is often called *incipient wilting*. Under such conditions the guard cells usually decrease in turgor as a result of osmotic movement of water into contiguous cells. Reduction in the turgor pressure of the guard cells as a result of the diminution of the volume of water in them will bring about a partial to complete closure of the stomates.

There is also some evidence that diminution in the water content of

the guard cells induces a decrease in the pH of their cell sap and a correlated conversion of sugar to starch. The resulting diminution in the osmotic pressure of the guard cells may lead to further loss of water from them into adjacent epidermal cells.

3. *The Temperature Factor in the Opening and Closing of Stomates.*—The results of various workers upon the influence of temperature on stomatal behavior are not in agreement and divergent views have been expressed upon the subject. According to Scarth (1932) relatively high temperatures (35-40° C.) accentuate opening of the stomates of *Zebrina pendula* in the light, and prevent closure or even induce opening in the dark. Relatively low temperatures (0-8° C.) prevent opening of the stomates of this species, even in the light. There is some evidence that the pH of the cell sap of the guard cells increases with rise in temperature and that this change in H-ion concentration is accompanied by hydrolysis of starch to sugar. Presumably the reverse transformation occurs at certain lower temperatures.

Daily Periodicity of Stomatal Opening and Closing.—The stomates of all species of plants which have been studied exhibit a more or less regular daily periodicity of opening and closing, although the behavior of the stomates upon plants of any one species varies, often markedly, depending upon the pattern of the daily cycle of environmental factors.

In the course of this book we shall have occasion to analyze the hour-to-hour variations of a number of physiological processes occurring in plants. Even in a given species the "daily periodicity"—as these hour-to-hour variations are termed—of any physiological process will vary greatly according to environmental conditions. It will therefore be convenient to choose a definite type of diurnal cycle of environmental factors as a reference standard in terms of which to discuss daily variations in the rate of various processes. For this purpose we shall select a "representative summer's day" as our criterion. We shall consider this hypothetical day to be characterized by a clear sky, a soil adequately well supplied with water (*i.e.* at approximately the "field capacity," Chap. XVI), and a maximum temperature of 30-35° C. (86-95° F.). Furthermore we will assume that the daily march of the environmental factors of solar radiation, air and soil temperatures, wind velocity, and atmospheric humidity will be representative of a day upon which the conditions as defined above prevail. In our subsequent discussion we shall refer to these as "standard day conditions" (Fig. 48). Such environmental conditions will actually be approximated on many summer days in temperate zone regions.

It is important to realize that all of the stomates on a plant do not open at the same time. Neither do all of them close at the same time. Under most conditions, however, stomates probably are more nearly coincident in the time

of their opening than in the time of their closing. The aggregate diffusive capacity of all of the stomates on a plant must therefore be thought of in terms of the two factors of the degree of opening of the individual stomates and the number of stomates that are open.

It is probable that the stomatal mechanism of every species of plant exhibits certain distinctive features in the way in which it reacts to various combinations of environmental factors; hence only broad generalizations can be formulated regarding the daily periodicity of stomatal behavior. The results of a number of investigators indicate that as a rule, under the condi-

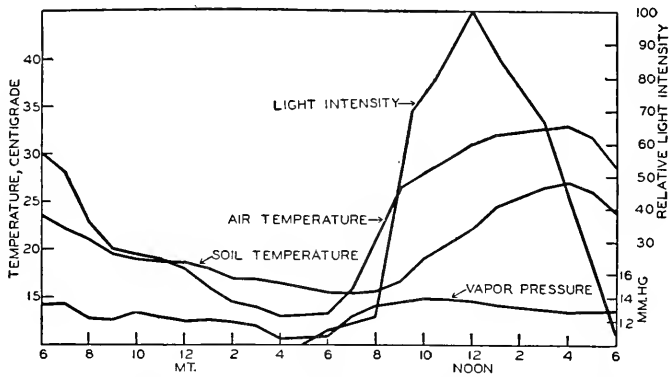

FIG. 48. Daily variation in certain environmental factors on a "standard day."

tions prevailing on a "standard day," the stomates of most mesic species of plants are open all or most of the daylight period and closed at night, their maximum diffusive capacity being attained about mid-day, or little before. The stomates open in the morning under the influence of the light factor and soon attain nearly their maximum diffusive capacity. Under "standard day" conditions, however, the water content and turgor of the leaf cells usually decrease progressively during most of the daylight period. Because of this internal water deficit which develops in the leaf, stomatal closure usually begins about noon or a little before. Virtually complete closure of the stomates often takes place considerably before the advent of darkness because of the predominant effect of the water factor over the light factor during the afternoon hours.

Innumerable other types of daily cycles of stomatal behavior are possible, a few of which will be described briefly. The diffusive capacity of the stomates sometimes rises to a mid- or late morning maximum, decreases markedly during the mid-day hours, rises to a secondary maximum during the afternoon,

and finally falls to a virtually zero value at approximately the termination of the daylight period. The stomates apparently behave in this way when a water deficit develops in the leaves somewhat earlier in the day than under "standard day" conditions. Partial closure of the stomates results during the morning hours. The resultant reduction in their diffusive capacity permits an increase in the water content of the leaf, and for a time the stomatal apertures again widen. Subsequently the water deficit of the leaf increases again, due to increased transpirational loss, and the stomates enter upon a second cycle of closing which continues throughout the remainder of the daylight period.

When the soil water supply is distinctly inadequate the stomates usually open incompletely and seldom remain open for the entire daylight period. Although with the advent of daylight the light factor favors stomatal opening, especially on clear days, the water content of the leaf is so low that opening is seldom complete. Furthermore the effect of the water factor usually begins to predominate over the light factor relatively early in the day, and stomatal closure may be complete by mid-day or even sooner. During prolonged droughts the stomates generally close progressively earlier and earlier each day and ultimately matinal opening may cease almost entirely.

On cloudy or rainy days, especially if the temperature is relatively low, the stomates of most species open less completely than on clear days when the soil is well supplied with water. This is due principally to the ineffectiveness of the light factor in inducing stomatal opening under such conditions. Hence opening of the stomates is incomplete and they do not remain open as long under such meteorological conditions as on a "standard day."

Nocturnal opening of the stomates has been reported for a number of species of plants (Loftfield, 1921, Desai, 1937, and others) but the conditions which result in this type of stomatal behavior are not clearly understood. Apparently it may be brought about by different combinations of environmental conditions and it seems likely that the conditions leading to nocturnal opening of the stomates may differ according to species. In the Norway spruce (*Picea excelsa*), for example, the stomates remain open during the night after a cloudy day on which relatively high temperatures have prevailed (Stålfelt, 1929.) There are some indications that high temperatures and a deficient water supply favor nocturnal opening of the stomates in some species. Scarth *et al.* (1933) have shown that a reduced partial pressure of oxygen in the atmosphere leads to stomatal opening in the dark. They suggest that nocturnal opening may be due to a reduced partial pressure of oxygen in the intercellular spaces resulting from night respiration. Finally

it should be noted that in some species such as maize it has never been possible to demonstrate night opening of the stomates.

Discussion Questions

1. Why is there usually little or no correlation between number of stomates per unit area of a leaf and its rate of transpiration?
2. Would you expect the amount of diffusion through a multiperforate septum to more nearly approach that through an open surface of the same area if the gradient of the diffusing gas is steep or if it is gradual?
3. Draw curves that might reasonably be expected to represent the daily variation in the "diffusive capacity" of the stomates (a) on a "standard day," (b) under otherwise "standard day" conditions but with the soil water content approaching the wilting percentage, (c) on a cloudy summer's day with the soil water content at approximately the field capacity.
4. Explain why benzene will penetrate rapidly into the intercellular spaces through open stomates when a leaf is brought in contact with it but water will not similarly penetrate unless applied with considerable force.
5. Suggest some possible explanations of the mechanism of night opening of stomates.
6. Stomatal closure sometimes occurs at times when no appreciable decrease takes place in the osmotic pressure of the guard cells. Explain.
7. Some authorities have considered that imbibitional swelling of colloidal materials in the guard cells may be an important factor in causing stomatal opening. What objections can be offered to this concept?

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CHAPTER XIV

FACTORS AFFECTING TRANSPIRATION

The rate of transpiration of a plant or any leaf on a plant varies from day to day, from hour to hour, and, frequently, from minute to minute. Variations in the rapidity with which water is lost by plants are due to the effects of environmental factors upon physiological conditions within the plant. The important environmental factors influencing the rate of transpiration are: (1) solar radiation, (2) humidity, (3) temperature, (4) wind, (5) soil conditions influencing the availability of water, and (6) atmospheric pressure. This last factor is relatively much less important than the other five listed. While the general effect of variations in the intensity or magnitude of each of these factors upon transpiration is well known, and has frequently been demonstrated by experimentation, the precise mechanism of the effect of each is not so easily amenable to experimental treatment. The following interpretation of the mechanism of the effects of these environmental factors upon the rate of transpiration is therefore a somewhat theoretical one, but is in accord with the experimental data available at the present time.

Solar Radiation.—This term refers to the visible light and other forms of radiant energy (infrared and ultraviolet radiations) reaching the earth from the sun (Chap. XIX). The principal effects of solar radiation upon transpiration result from the influence of light upon the opening and closing of the stomates. In most of the species of plants which have been studied the stomates are usually closed in the absence of light, thus causing a virtually complete cessation of stomatal transpiration during the hours of darkness. Since none of the other environmental factors can have any influence upon stomatal transpiration except when the stomates are open, light occupies a position of prime importance among the environmental conditions influencing transpiration.

A second important effect of solar radiation upon transpiration operates through its influence on leaf temperatures. In direct sunlight the temperature of leaves is almost invariably higher than the air temperature. This effect will be analyzed later in this chapter.

Humidity.—Several units are used for designating the humidity conditions of an atmosphere. One of these is the *actual* vapor pressure of the atmosphere. This should not be confused with the *saturation* vapor pressure (Chap. XII). Since the rates of diffusion and evaporation are influenced directly by the vapor pressure of the atmosphere this is usually the most satisfactory unit in which to express humidity values for physiological purposes.

The *vapor pressure deficit* is also a common, and for many purposes a valuable unit for the expression of humidity values (Anderson, 1936). The vapor pressure deficit is the difference between the actual vapor pressure of the atmosphere and the vapor pressure of a saturated atmosphere at the same temperature. As an example, consider an atmosphere having a vapor pressure of 10.51 mm. Hg at 25° C. The saturation vapor pressure of this atmosphere would be 23.76 mm. Hg (Table 23). Hence its vapor pressure deficit is 13.25 mm. Hg (23.76 − 10.51).

If an evaporating surface and the atmosphere are both at the same temperature the vapor pressure deficit indicates directly the steepness of the vapor pressure gradient between the atmosphere and the evaporating surface. The term vapor pressure gradient as applied to water-vapor has the same significance as the term diffusion pressure gradient which has been frequently used in previous discussions. In fact the vapor pressure gradient of water vapor *is* its diffusion pressure gradient. As long as the temperature of the evaporating surface does not differ materially from that of an atmosphere the rate of evaporation from a saturated surface into that atmosphere will show a close proportionality to its vapor pressure deficit. However, the temperature of an evaporating surface is seldom exactly the same as that of the atmosphere, and the deviation in temperature between the two is often very considerable. There are therefore many situations in which rates of evaporation do not show a close proportionality with the vapor pressure deficit of the surrounding atmosphere.

The most generally employed of all units in expressing humidity values is the *relative humidity,* which is the percentage saturation of an atmosphere at a given temperature. For example, a saturated atmosphere at 30° C. will have a vapor pressure of 31.82 mm. Hg. The relative humidity of this atmosphere would be 100 per cent. If only half the amount of vapor is present that would be present at saturation at this temperature (*i.e.* a vapor pressure of 15.91 mm. Hg) then the relative humidity would be 50 per cent, etc. (Table 23). The relative humidity depends both on the concentration of water-vapor in the atmosphere and upon the temperature. Any increase in temperature with no accompanying change in the amount of water-vapor present always results in a decrease in relative humidity, because an increase

in temperature means that a higher vapor pressure will be required before the atmosphere is saturated.

Expression of humidity values in terms of relative humidity, although a common practice, is unsatisfactory for physiological purposes because the same relative humidity, 50 per cent for example, may refer to widely different vapor pressures and vapor pressure deficits (Table 23). A relative humidity of 50 per cent at 10° C. is equivalent to a vapor pressure deficit of 4.60 mm. Hg, while a relative humidity of 50 per cent at 50° C. is equivalent to a vapor pressure deficit of 46.26 mm. Hg. In other words, with the same relative humidity, evaporation from moist surfaces exposed to the air will be many times as rapid at 50° C. as at 10° C. Only when all of the relative humidity values are recorded at the same temperature are they an expression of the relative differences in the vapor pressure of an atmosphere. The general relations between relative humidity, vapor pressure, and vapor pressure deficit are summarized in Table 23.

TABLE 23—THE RELATION BETWEEN RELATIVE HUMIDITY, VAPOR PRESSURE DEFICIT AND VAPOR PRESSURE AT DIFFERENT TEMPERATURES

Temperature		Actual vapor pressure (mm. Hg) at indicated relative humidity (Read down)										
°C.	°F.	0	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
0	32	0	0.46	0.92	1.37	1.83	2.29	2.75	3.21	3.66	4.12	4.58
5	41	0	0.65	1.31	1.96	2.62	3.27	3.92	4.58	5.23	5.89	6.54
10	50	0	0.92	1.84	2.76	3.68	4.60	5.53	6.45	7.37	8.29	9.21
15	59	0	1.28	2.56	3.84	5.12	6.40	7.67	8.95	10.23	11.51	12.79
20	68	0	1.75	3.51	5.26	7.02	8.77	10.52	12.28	14.03	15.79	17.54
25	77	0	2.38	4.75	7.13	9.50	11.88	14.26	16.63	19.01	21.38	23.76
30	86	0	3.18	6.36	9.55	12.73	15.91	19.09	22.27	25.46	28.64	31.82
35	95	0	4.22	8.44	12.65	16.87	21.09	25.31	29.53	33.74	37.96	42.18
40	104	0	5.53	11.06	16.60	22.13	27.66	33.19	38.72	44.25	49.79	55.32
45	113	0	7.19	14.38	21.56	28.75	35.94	43.13	50.32	57.50	64.69	71.88
50	122	0	9.25	18.50	27.75	37.00	46.26	55.51	64.76	74.01	83.26	92.51
		100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	0%
		Vapor pressure deficit (mm. Hg) at indicated relative humidity (Read up)										

In general the greater the vapor pressure of an atmosphere, other factors remaining unchanged, the slower the rate of transpiration. The rate of diffusion of water-vapor out of a leaf depends upon the difference between the vapor pressure in the intercellular spaces and the vapor pressure of the outside atmosphere, since the vapor pressure is a measure of the diffusion pressure

of the water-vapor. Let us suppose that the vapor pressure of the intercellular spaces is 31.82 mm. Hg which is the value for a saturated atmosphere at 30° C. Such a vapor pressure is frequently attained in the internal air spaces of leaves. Let us further assume that at the same time the vapor pressure of the atmosphere is only half as great (15.91 mm. Hg). Such a value would be a representative one for a warm summer's day in the eastern United States. In very quiet air the vapor pressure in the neighborhood of transpiring leaf surfaces may be greater than that in the atmosphere in general but in this discussion it is assumed that there is sufficient air movement to prevent any appreciable accumulation of water-vapor in the vicinity of the leaves.

Under the conditions as stated diffusion of water-vapor would occur through the stomates at a relatively rapid rate. If the vapor pressure of the atmosphere were lowered below this value the rate of diffusion of water-vapor out of the leaf would be increased; conversely increase in the vapor pressure of the atmosphere would result in a decrease in the diffusion rate of water-vapor out of the leaf. Similarly, an increase in the vapor pressure of the intercellular spaces would result in an increase in the rate of transpiration while a decrease in the vapor pressure of the intercellular spaces relative to that of the atmosphere would have the converse effect. On the rare occasions when the vapor pressures of the atmosphere and of the intercellular spaces are equal, no transpiration will occur, even if the stomates are open.

The final statement in the preceding paragraph, should not, however, be interpreted to mean that transpiration can never occur into a saturated atmosphere. If the temperature of the leaf is higher than that of the surrounding atmosphere this will, if the intercellular spaces be saturated with water-vapor, result in a higher vapor pressure in them than in the atmosphere, even when the latter is also saturated with water-vapor. Under such conditions outward diffusion of water-vapor occurs, resulting in a localized region of supersaturated atmosphere around the leaf in which condensation of water-vapor may take place.

Temperature Effects on Transpiration. — 1. *Thermal Relations of Leaves.*—While leaf temperatures often do not deviate greatly from surrounding atmospheric temperatures the discrepancy between the two is frequently sufficiently great to make it necessary to take it into account in careful experimental work. Leaves exposed to direct solar or artificial radiation usually have temperatures of from 2 to 10° C. (sometimes even more) in excess of that of the atmosphere. Under other conditions, to be described later, leaf temperatures may be less than that of the atmosphere.[1]

[1] Leaf temperatures are generally measured by means of thermocouples. A thermocouple is made by twisting together the ends of two fine wires of dissimilar

Theoretically the temperature of a leaf may be regarded as conditioned by four different influences: (1) thermal absorption, (2) thermal emission, (3) internal endothermic (energy-storing) processes, such as photosynthesis and transpiration, and (4) internal exothermic (energy-releasing) processes such as respiration. The influence of this last factor upon leaf temperatures is practically always negligible and will be disregarded. (*Cf.* Chap. XXIX for examples in which internally produced heat of respiration does influence the temperature of plant organs.) Similarly the quantity of energy transformed in photosynthesis is relatively so small that it need not be considered in evaluating the factors determining the temperature of leaves.

Thermal emission refers to the loss of heat from a leaf by the processes of conduction, convection, and radiation (Brown and Escombe, 1905). *Thermal absorption* refers to the gain of energy by a leaf by these same physical processes.

Heat transmission which is brought about by intermolecular contacts is known as (thermal) conduction. The greater the temperature difference between a leaf and its environment the more rapidly conduction of heat will occur from the leaf to the gases of the atmosphere, if its temperature is the higher, or in the opposite direction if the temperature of the atmosphere is the higher.

Whenever loss of heat is occurring by conduction from a leaf to adjacent gas molecules of the atmosphere convection currents (Chap. VII) are set up in the atmosphere in the vicinity of the leaf. Cooler gas will displace the gas in the vicinity of the leaf surfaces which has become warmed as a result of thermal conduction from the leaf. This accelerates the rate at which conduction can occur from the leaf.

Radiation is the transfer of radiant energy across space. The best known types of radiant energy are light, infrared radiations ("heat waves") and ultraviolet radiations. Radiant energy is commonly pictured as being propagated across space in the form of undulatory waves (see Chap. XIX for discussion of another concept of the nature of radiant energy). Radiation occurs from the molecules of one body to those of another only if the radiating body is at a higher temperature than the receiving body. Thus light, infrared and ultraviolet radiation are transferred from the sun to the earth.

metals, for example copper and constantan (an alloy of copper and nickel). A difference of electrical potential is set up between two wires thus brought into intimate contact. The magnitude of this electrical potential is very nearly directly proportional to the temperature of the thermocouple. In actual practice two junctions are generally used, one being inserted into the leaf blade, the other being kept at a standard temperature, usually 0° C. The difference in potential between the two thermocouples is generally measured with a potentiometer.

In like manner a warm stove loses heat by invisible infrared radiation to its environment. Radiation occurring from leaves is also in the infrared range of wave lengths.

The temperature of a leaf exposed to direct sunlight or strong artificial illumination almost invariably exceeds that of the atmosphere. Thermal absorption—in this case direct absorption of solar radiations—proceeds under such conditions at a rapid rate. A portion of the absorbed radiant energy is dissipated (usually) by transpiration, and a portion is lost from the leaf by thermal emission. As already shown in Chap. XII transpiration alone is never adequate to dispose of the energy absorbed if the leaves are exposed to strong insolation. Thermal emission under these conditions will involve loss of heat both by radiation and by conduction. In general the greater the excess of leaf temperature over that of the atmosphere the larger the proportion of heat loss by thermal emission as contrasted with transpiration (Watson, 1934).

On a cloudy day the temperature of leaves seldom deviates very greatly from that of the enveloping atmosphere. Heat exchanges between a leaf and its environment under such conditions probably occur principally by conduction. Similarly at night leaf temperatures usually do not deviate greatly from those of the surrounding atmosphere.

Leaves sometimes have a lower temperature than the surrounding atmosphere. This is often true, for example, of leaves which are transpiring fairly rapidly but which are not exposed to direct sunlight. Such leaves are often cooler by several degrees centigrade than the atmosphere. It is also possible that leaves sometimes lose heat energy by direct radiation to their environment rapidly enough to result in a lowering of their temperature below that of the atmosphere (Curtis, 1936a). This is especially likely to occur on clear nights when the vapor pressure of the atmosphere is low. These conditions favor direct radiation from the leaves to the sky, *i.e.* to relatively cold gases, especially carbon dioxide and water-vapor.

The temperature of leaves usually fluctuates from minute to minute, especially during daylight hours. Minor fluctuations are due largely to shifts in wind velocity. The thermal emissivity of leaves exposed to direct sunlight is invariably increased by an increase in wind velocity. When the sunlight is intermittent, frequent changes in leaf temperatures also occur. Each time the sun is obscured by a cloud a sudden drop in leaf temperature usually takes place. Contrariwise, when the sun emerges from behind a cloud there is usually a distinct and abrupt rise in leaf temperature.

The factors controlling the internal temperatures of the other organs of plants are in general similar to those influencing leaf temperatures. The temperatures of fleshy leaves, fruits, tree trunks, succulent stems such as those

of cacti, etc. under the influence of direct insolation may often greatly exceed those of the surrounding atmosphere. The side of an apple fruit exposed to direct sunlight, for example, may have a temperature of from 12 to 25° C. higher than the air temperature (Brooks and Fisher, 1926).

2. *The Influence of Temperature upon Transpiration Rates.*—The effects of temperature upon the rate of stomatal transpiration can be most clearly analyzed in terms of its effect upon the difference in vapor pressures between the intercellular spaces and the outside atmosphere (Renner, 1915). In this part of the discussion it is again assumed that the air movement is sufficient to prevent any appreciable accumulation of water-vapor in the vicinity of the leaf surfaces. Suppose that the temperature of both the leaf and the surrounding atmosphere increases from 20° C. to 30° C. Unless the leaf is markedly deficient in water this will result in an increase in the vapor pressure of the intercellular spaces from approximately 17.54 mm. Hg to approximately 31.82 mm. Hg, these being the values for a saturated atmosphere at 20° C. and 30° C., respectively. The atmosphere of the leaf intercellular spaces is in direct contact with the relatively extensive evaporating surface of the mesophyll cell walls, hence the vapor pressure in the intercellular spaces tends to remain in equilibrium with the water in the mesophyll cells. In order to simplify this part of the discussion, it will be assumed that the atmosphere of the leaf intercellular spaces maintains essentially a saturation vapor pressure for the prevailing leaf temperature. Under certain conditions, as shown later in this chapter, maintenance of even an approximately saturated atmosphere throughout the intercellular spaces probably does not occur, but for the time being this possibility will be disregarded.

In the surrounding atmosphere, however, vapor pressure conditions are very different. On clear days, that is, on the very type of day upon which the highest rates of transpiration occur, there is frequently little change in the vapor pressure of the atmosphere over land surfaces during the course of a single day.[2] Evaporation into the atmosphere is insufficient to permit a rapid building up of the vapor pressure towards the value for a saturated atmosphere as the temperature of the air increases during the day. It might be thought

[2] This statement should not be misinterpreted to read that the vapor pressure of the atmosphere is invariable. The magnitude of the vapor pressure of the atmosphere varies greatly from day to day and from season to season, depending upon the prevailing climatic conditions. On cloudy or rainy days, the atmospheric vapor pressure is generally greater than on clear days during the same season; in the summer months it is generally greater than in the winter months, etc. Nevertheless the statement made above that on clear, bright days there is *often* little change in the vapor pressure of the atmosphere is essentially correct (Day, 1917).

that the process of transpiration itself, occurring on a grand scale from a vegetation-covered area of the earth's surface would be sufficient to increase the vapor pressure of the lower layers of the atmosphere during the daylight hours. The atmosphere is so vast, however, in relation to the amount of water-vapor lost by plants, that over short periods, transpiration has only a slight effect on its vapor pressure except in deep ravines or other local habitats in which movement of the air is restricted.

Increase in the temperature of the atmosphere does result in an increase in the speed of the water-vapor molecules present. If the volume of the atmosphere remained constant this would result in a small increase in its vapor pressure. But even this effect is never fully realized in the atmosphere because an increase in temperature also results in an expansion of the atmosphere, entirely or largely offsetting its influence in increasing vapor pressure.

If we assume for the purpose of our specific example that the vapor pressure of the atmosphere at 20° C. was half that of a saturated atmosphere at that temperature—8.77 mm. Hg—then the excess vapor pressure of the leaf over that of the atmosphere at 20° C. was 8.77 mm. Hg (17.54 − 8.77). At 30° C., however, the vapor pressure of the intercellular spaces would have increased to about 31.82 mm. Hg while the increase in the vapor pressure of surrounding atmosphere would usually be so slight that it can be disregarded. The excess vapor pressure of the intercellular spaces over the atmosphere is now 23.05 mm. Hg (31.82 − 8.77) which will result in diffusion of water-vapor out of the leaf at a rate nearly three times as fast as at 20° C. The effect of a rise in temperature therefore is principally an increase in the steepness of the diffusion gradient (vapor pressure gradient) of water-vapor through the stomates, and hence an increase in the rate of transpiration.

So far we have considered only examples in which the temperature of a leaf and the surrounding atmospheres are the same. We have already noted, however, that the temperature of leaves exposed to direct sunlight is almost always higher than that of the atmosphere. If the temperature of a leaf is increased above that of the surrounding atmosphere by the absorption of solar radiation, the usual effect is an increase in the magnitude of the excess vapor pressure of the intercellular spaces over that of the outside atmosphere. Let us continue the example which has been presented earlier. At 30° C. under the conditions as stated the vapor pressure difference between the intercellular spaces and the atmosphere was about 23.05 mm. Hg. Suppose, however, that due to the absorption of radiant energy, the temperature of the leaf is 35° C. while that of the atmosphere remains at 30° C. Water would evaporate from the walls of the mesophyll cells until the vapor pressure in the inter-

cellular spaces approximates that of a saturated atmosphere at 35° C. (42.18 mm. Hg). The gradient between the intercellular spaces and the atmosphere is therefore increased to about 33.41 mm. Hg (42.18 − 8.77) and the rate of transpiration is correspondingly increased. The sudden increases in transpiration rate which are often observed when plants are shifted from shade to direct sunlight, as for example in potometer experiments, are undoubtedly due largely if not entirely to effects of this type. Similarly it has been observed that a sudden decrease may occur in the transpiration rate when a passing cloud obscures the sun, which is followed by just as sudden an increase when the sun again emerges from behind the cloud. For further analysis of the effects of leaf temperature upon transpiration rates see Curtis (1936b).

Wind.—The effect of wind upon the rate of transpiration is far from simple, and depends in part upon the other prevailing environmental conditions. Usually, however, increase in wind velocity, within limits, results in an increase in the rate of transpiration (Wrenger, 1935). This is usually explained by assuming that water-vapor often accumulates in the vicinity of transpiring leaves in a quiet atmosphere, especially if they are not exposed to direct sunlight. The result of such an accumulation of water-vapor is a decrease in the steepness of the vapor pressure gradient through the stomates and hence a decrease in the rate of transpiration. If, however, the leaves are exposed to a wind, any accumulation of water-vapor molecules in the immediate vicinity of the leaf surfaces will be dispersed. The effective result will be an increase in the steepness of the vapor pressure gradient through the stomates, and a consequent increase in the rate of loss of water-vapor.

Whenever a temperature differential exists between a leaf and the surrounding atmosphere, convection currents are set up in the gases in the vicinity of a leaf which may largely or entirely prevent any accumulation of water-vapor in the immediate vicinity of the leaf. The influence of wind in raising the transpiration rate of leaves is probably more effective, therefore, when they are subjected to such conditions that their temperature does not depart appreciably from that of the surrounding atmosphere.

The effect of wind in dispersing accumulated water-vapor in the vicinity of the leaf surfaces is probably of less importance in its influence upon transpiration rates than its effect in causing the swaying of branches and shoots, and the bending, twisting, and fluttering of leaf blades. It has been shown experimentally that immobile leaves usually transpire less than similar leaves allowed to bend and move freely when both are exposed to wind of equal velocity. Such bending and contortion of leaves may increase the rate of water-vapor loss in part by compressing the intercellular spaces, thus forcing water-vapor and other gases out through the stomates.

A gentle breeze is relatively much more effective in increasing the transpiration rate than winds of greater velocity (Fig. 49). Winds of very high velocity have been observed to have a retarding effect upon transpiration. This is probably due to closure of stomates under such conditions.

The effect of wind upon the transpiration of a leaf which is exposed to intense insolation is further complicated by the fact that a wind may have a cooling effect upon the leaves, due to their increased thermal emissivity under such conditions. Reduction in the temperature of a leaf, as we have already seen, exerts a decelerating effect upon transpiration. If conditions are such

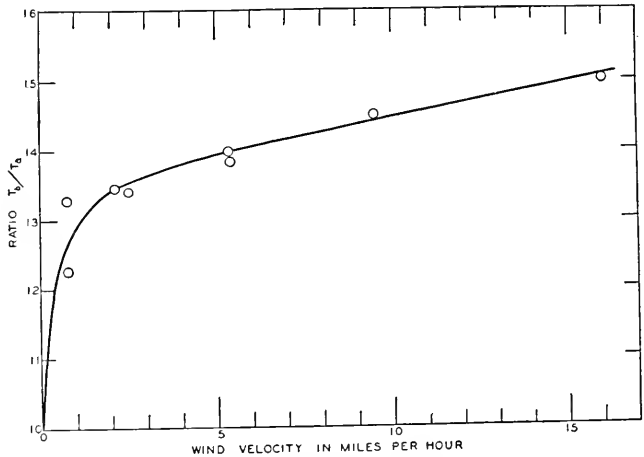

FIG. 49. Relation between wind velocity and rate of transpiration of sunflower plants expressed as ratio between plants in wind (T_b) and plants in quiet air (T_a). Data of Martin and Clements (1935).

that this cooling effect of wind is predominant increased wind velocity may result in a diminution in transpiration rate.

Soil Conditions Influencing the Availability of Water.—Although transpiration can continue for short periods at rates considerably in excess of the rate of absorption of water (Chap. XVIII), in general, if soil conditions are such that absorption of water is appreciably retarded, the rate of transpiration will soon show a corresponding retardation. The availability of soil water to the plant is therefore an important and, in fact, often the limiting factor in transpiration. The principal soil factors which affect the rate of absorption of water by plants are: (1) available soil water, (2) soil temperature, (3) aeration of the soil, and (4) concentration of solutes in the soil solution (Chap. XVII). All of these factors indirectly influence the rate of transpiration.

Atmospheric Pressure.—It has been demonstrated experimentally that a reduction in atmospheric pressure results in an increase in the rate of transpiration (Sampson and Allen, 1909). This result would be predicted theoretically since reduction in the density of the atmosphere would be expected to permit diffusion of water-vapor to occur into it more rapidly. In any given locality variations in atmospheric pressure are too slight to have any significant effect upon the rates of transpiration. Plants growing at high altitudes are subjected to distinctly lower atmospheric pressures than the plants of lowlands, and in comparative evaluations of the transpiration rates of species growing in these two types of habitats the influence of this factor must be considered.

Structural Features of Plants which Influence the Rate of Transpiration.—A number of diverse species of plants growing under identical environmental conditions usually differ very greatly in transpiration rates. Physiological factors, such as differences in stomatal behavior, cell sap concentration, colloid content of the cells, etc. are undoubtedly partially responsible for the fact that all plants do not lose water-vapor at the same rate even under the same environmental conditions. Structural differences, particularly in the leaves, also account in part for unlike rates of transpiration of different species growing in the same environment. Many of the supposed effects of structural differences in plants upon their transpiration rates are, however, based solely on inferences, and not upon experimentally determined facts. Many species which on the basis of their anatomy have been judged to have a low transpiration rate later were actually found to transpire very rapidly when environmental conditions were suitable. The following discussion of the anatomical features known or generally believed to influence the rate of transpiration will therefore be very brief.

1. *Thickness of the Cutin.*—It can be easily demonstrated that water evaporates more readily from uncutinized epidermal cell walls than from those which are coated with a layer of cutin. It is sometimes assumed, therefore, that the thicker the layer of cutin on the outer walls of the epidermis of a leaf, the slower the rate of *cuticular transpiration* from that leaf. Actually, however, it seems obvious that any increased thickness in the cutin layer beyond that at which cuticular transpiration becomes negligible will have no appreciable influence on the rate of evaporation from the epidermal cell walls.

2. *Epidermal Hairs.*—Hairs are prominent features of one or both of the surfaces of the leaves of many species. *Living* hairs probably increase the rate of *cuticular transpiration* by increasing the exposed surface of the leaf. Many botanists have assumed that *dead* hairs reduce the rate of stomatal transpiration, particularly under conditions of intense sunlight or strong

winds. In the former case it has been supposed that the whitish hairs reflect such a large proportion of the incident light that the leaf temperature is not as high as it otherwise would be, and that the rate of transpiration is correspondingly lower. In the latter case it has been supposed that the hairs impose a mechanical barrier to the effect of wind on transpiration. However, Sayre (1920) found that removal of the hairs from the leaves of the mullein (*Verbascum thapsus*) had little effect upon the rate of stomatal transpiration under ordinary conditions of light intensity and wind velocity, although such treatment did result in an increase in cuticular transpiration.

3. *Ratio of Internal to External Surface in Leaves.*—Although transpiration rates are most frequently expressed in terms of the exposed area of the leaf surface, most of the water-vapor lost from leaves evaporates from the walls of the mesophyll cells which bound the intercellular spaces. The area of this internal evaporating surface in proportion to the external surface of a leaf varies greatly not only in leaves of one species as compared to those of another, but in leaves of the same species if they have developed under different environmental conditions. Computations of the ratio of internal exposed surface to external exposed surface of leaves of a number of species have yielded values ranging from 6.8 to 31.3 (Turrell, 1936). In general the greater the proportion of palisade to sponge tissue the greater this ratio. The thickness of the leaf will also obviously be a factor influencing this ratio. The ratio is greater in sun leaves than in shade leaves of the same species; for the lilac the values are 13.2 and 6.8, respectively.

It has generally been supposed that the transpiration rates of leaves in which a relatively large area of mesophyll cell walls is exposed to the intercellular spaces as compared with the exposed epidermal area of the leaf are greater than in leaves in which the contrary condition obtains. Theoretically such an effect could be exerted only if the area of exposed mesophyll cell walls exercised a controlling influence upon the steepness of the vapor pressure gradient through the stomates. When transpiration is relatively high a steeper vapor pressure gradient may be maintained by leaves possessing relatively extensive internal evaporating surfaces than by those which do not. The higher transpiration rate of sun leaves as compared with shade leaves on the same plant, which has been observed in some species, may be partly explainable on this basis.

4. *Sunken Stomates.*—The stomates of many species of plants are sunken below the general level of the epidermis (Fig. 42, *D*). Since the length of a diffusion gradient is one of the factors governing its steepness, diffusion through a small opening is slower if the gas must pass through a relatively long tube before reaching the orifice, than if the diffusion is through a shorter

tube. In leaves in which the stomates are not sunken the diffusion column is relatively short, amounting only to the diameter of the guard cells as seen in cross section, while in sunken stomates the column of gas may be longer by several times. The diffusion of gases through such stomates is undoubtedly slower than through stomates of the ordinary type. This retarding effect of sunken stomates upon the rate of transpiration as compared with the stomates of the non-sunken type has been estimated by Renner (1910) to range from 30 to 70 per cent.

5. *Structure and Distribution of the Root System.*—Under some environmental conditions an individual plant of a given species will develop a greater leaf surface in proportion to the extent of its root system than under others. The latter type of structural development will, other conditions being equal, favor the maintenance of higher transpiration rates than the former.

The distribution of a root system in the soil sometimes influences rates of water absorption and transpiration. In a habitat where deep-rooted and shallow-rooted species are growing side by side the former may transpire more rapidly during drought periods than the latter because their roots penetrate to a soil horizon which still contains available water, while roots of the latter are in a moisture deficient soil.

The completeness with which the soil mass is interpenetrated by roots may also influence rates of absorption and transpiration. Thus Miller (1916) found that sorghum plants have almost twice as many fibrous roots as maize plants. Rates of absorption and transpiration can be better maintained, especially when the soil is relatively dry, by plants with the sorghum type of root system than by plants with the maize type of root system.

The Daily Periodicity of Transpiration.—All plants exhibit a daily periodicity of transpiration rate which varies somewhat with the species and is greatly influenced by the environmental conditions to which the plant is exposed. We shall first consider the transpiration periodicity upon a "standard day" as defined in the preceding chapter, since most experiments upon daily variations in transpiration rate have been conducted upon plants growing under approximately such conditions. It should not be assumed, however, that under natural conditions the daily periodicity of transpiration usually or "normally" follows the trend shown in Fig. 50. The daily periodicity of transpiration under these roughly defined conditions does, however, afford a convenient reference standard with which to compare the many variations which are manifested by this phenomenon.

The transpiration rate during the hours of darkness is generally low, and in most species water loss during this period may be regarded as almost

entirely cuticular. It is not justifiable to assume that absolutely no stomatal transpiration occurs at night in any species unless this is definitely known to be true. In some species of plants, as we have already seen, night stomatal opening is a common occurrence, while in others it occurs under certain environmental conditions. Even in those species in which the stomates are normally closed during the hours of darkness, a few may remain open.

The transpiration rate shows a rapid rise during the early morning hours, followed by a slower but consistent increase which culminates in a point of maximum transpiration which occurs sometime during the mid-day hours (11 A.M. - 2 P.M.). Following this peak of transpiration, the rate decreases, usually consistently although often with an accelerated rate in the late after-

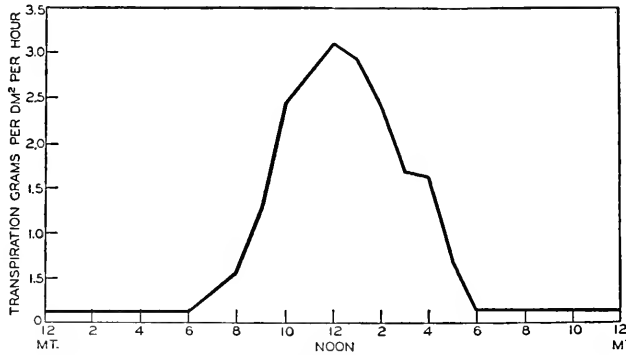

FIG. 50. Daily periodicity of transpiration in mullein (*Verbascum thapsus*). Data of Sayre (1920).

noon, until the low and virtually steady night rate is attained at approximately the termination of the daylight period.

The daily periodicity in the rate of stomatal transpiration in most species of plants can be interpreted almost entirely in terms of two variable factors: (1) the diffusive capacity of the stomates, and (2) the vapor pressure gradient between the intercellular spaces of the leaf and the outside atmosphere. In analyzing the daily periodicity of transpiration it is the diffusive capacity of the stomates in the aggregate (*i.e.* all of the stomates on a plant) which must be considered. The aggregate diffusive capacity of the stomates on a plant on which all of the stomates were half open, for example, would be much greater than that for a similar plant on which half of the stomates were fully open and half fully closed.

With the advent of daylight the stomates open over a period of time which varies in length according to the species and with environmental conditions. Some open earlier or more rapidly than others. The initial rise in the rate of

transpiration in the morning is due to the opening of the stomates, resulting in a gradual increase in their aggregate diffusive capacity. As each stomate opens a vapor pressure gradient is established through it between the atmosphere of the intercellular spaces and the outside atmosphere. During the hours of darkness the leaf cells regain most or all of the water which they have lost during the preceding day, and thus attain approximately their normal turgidity. This facilitates the evaporation of water into the intercellular spaces, so that when the stomates open in the morning the internal atmosphere of the leaf is usually saturated with water-vapor, or practically so. The vapor pressure of the intercellular spaces is therefore at the maximum possible for that leaf temperature. This is, on clear days, almost always in excess of the vapor pressure of the atmosphere when the stomates open, and often considerably so. Hence outward diffusion of water-vapor through the stomates usually begins as soon as they are open.

Once a stomate is well open, minor variations in the size of the stomatal aperture have little or no effect on the rate of water-vapor loss through it. The rate of transpiration continues to increase, however, for some time after the stomates, in the aggregate, have attained their maximum diffusive capacity. This is due to a gradual increase in the steepness of the vapor pressure gradient through the stomates. As the day progresses the temperature of both the atmosphere and the leaf increases; if the latter is in direct sunlight its temperature is invariably somewhat in excess of that of the atmosphere. On clear days, as previously described, the vapor pressure of the intercellular spaces usually increases relative to that of the atmosphere with a rise in temperature. The steepness of the vapor pressure gradient between the internal atmosphere of the leaf and the external atmosphere therefore usually increases progressively during the earlier part of the day, and this is the important factor accounting for a rise in the transpiration rate once the stomates have attained approximately their maximum diffusive capacity.

Almost from the moment when the stomates begin to open in the morning, a train of events is set in operation in the plant which ultimately causes a reduction in the rate of transpiration. During approximately the first half of the day, however, these factors are more than offset by the factors resulting in an increase in the rate of transpiration. In most plants, while transpiration is occurring rapidly, the rate of absorption of water does not keep pace with the rate at which water-vapor is lost from the leaves. This results in a reduction in the water content of the entire plant, and especially that of the leaves. In more extreme cases wilting results, but under standard day conditions the leaves seldom pass beyond the stage of *incipient wilting* (Chap. XVIII), which corresponds only to a partial loss of turgor by the leaf cells.

Decrease in the water content of the leaf cells also results in a rise in their diffusion pressure deficit, and a correlated decrease in their vapor pressure. A fully turgid cell (*i.e.* one with a diffusion pressure deficit of zero) has an equilibrium vapor pressure equivalent to that of pure water regardless of its osmotic pressure. At 20° C. this is 17.54 mm. Hg. If the diffusion pressure deficit of the mesophyll cells of a leaf at this temperature were raised to 100 atmos., a value which can be attained in only a very few species of plants, their vapor pressure would decrease by only about 1.27 mm. Hg (Table 24). Since the vapor pressure of the cell walls, with which the vapor pressure of the intercellular spaces tends to be in equilibrium, in turn tends to be in equilibrium with that of the cell sap, it would appear that an increase in the diffusion pressure deficit of the mesophyll cells has only a very slight influence upon vapor pressure conditions in the intercellular spaces.

TABLE 24—RELATION BETWEEN DIFFUSION PRESSURE DEFICIT AND VAPOR PRESSURE OF WATER AT 20° C.

Diffusion pressure deficit	Relative humidity	Vapor pressure	Diffusion pressure deficit	Relative humidity	Vapor pressure
Atmos.	Per cent	Mm. Hg	Atmos.	Per cent	Mm. Hg
0	100	17.54	155	89	15.61
13.4	99	17.36	171	88	15.44
26.9	98	17.19	186	87	15.26
40.6	97	17.01	201	86	15.08
54.5	96	16.84	217	85	14.91
68.4	95	16.66	233	84	14.73
82.4	94	16.49	249	83	14.56
96.7	93	16.31	265	82	14.38
111	92	16.14	281	81	14.21
125	91	15.96	298	80	14.03
140	90	15.79	315	79	13.86

However, certain other factors must be taken into consideration in evaluating the effect of a diminished leaf water content upon the vapor pressure of the intercellular spaces. Decrease in the water content of the leaf cells involves a reduction in the quantity of water in the cell walls as well as in other parts of the cell. Water undoubtedly passes through cell walls principally through the intermicellar material. The principal effect of a decrease in the quantity of water in the walls is a shrinkage in the volume of the hydrophilic intermicellar material and a resulting contraction in diameter of the intermicellar capillaries. This probably results in a decrease in the permeability of the walls to water. Hence, under such conditions, water often

may not pass across the walls with sufficient rapidity that an equilibrium vapor pressure is maintained between the cell sap and the outer surface of the cell walls. When the water content of a leaf is low the vapor pressure of the cell wall surface in contact with the intercellular spaces may therefore be less than the vapor pressure of the water in the cell sap. Any such reduction in the vapor pressure of the intercellular spaces results in decreasing the steepness of the vapor pressure gradient between the intercellular spaces and the outside atmosphere, and hence decreases the rate of transpiration. The experimental results of Thut (1938) indicate that the vapor pressure of the intercellular spaces is often at less than a saturation value.

During the course of a day, however, the steepness of the vapor pressure gradient through the stomates may be decreased in still another and perhaps more important way. When the stomates open in the morning the vapor pressure gradient between the saturated internal leaf atmosphere and the outside atmosphere is short and hence steep, being approximately equal in length to the depth of the guard cells. For some time after the stomates open essentially such a condition is probably maintained. As the day advances the rapid loss of water-vapor molecules out of the intercellular spaces, perhaps coupled with a gradual reduction in the vapor pressure of the cell walls, makes it less and less likely that a condition of saturation can be maintained throughout the intercellular spaces. The zone in which a vapor pressure in equilibrium with the water in the mesophyll cell walls is maintained almost certainly retreats more and more deeply into the intercellular spaces. Eventually it may be restricted to a thin layer just above the evaporating surfaces of the mesophyll cell walls. This gradual lengthening of the vapor pressure gradient through the stomates is probably an important factor in bringing about a reduction in the rate of transpiration during the afternoon hours.

It has frequently been observed in studies of transpiration periodicity that the transpiration rate often begins to decrease during the mid-day period before any appreciable change occurs in the area of the stomatal apertures. This indicates that the initial reduction in transpiration rate is induced by some other factor than a change in the diffusive capacity of the stomates. As the preceding discussion has shown this other factor is almost certainly a reduction in the steepness of the vapor pressure gradient through the stomates.

By late afternoon the air temperature and the intensity of the solar radiation begin to decrease appreciably, thus inducing a decrease in the temperature of the leaf. This lowering of the leaf temperature may further decrease the vapor pressure of the intercellular spaces, and hence further depress the steepness of the vapor pressure gradient, since temperature changes have very little influence on the vapor pressure of the outside atmosphere under "stand-

ard day" conditions. Thus the effect of a reduction in the vapor pressure gradient through the stomates in decreasing the rate of transpiration, which first becomes apparent during the mid-day period, continues with augmented effect as the hours of darkness approach.

Continued diminution in the leaf water content due to the excess of transpiration over absorption eventually results also in a diminution of the turgor of the guard cells due to a decrease in the water content of the leaf. This results in a gradual closure of the stomates. Some of the stomates on a plant probably begin to close even before the time at which the peak of the transpiration rate is attained. In all likelihood stomates near the margin or tip of a leaf begin to close before those in the middle, since the effects of deficiency of water usually appear first in these regions of a leaf. With an increasing leaf water deficit more and more of the stomates on the plant close. This soon results in a reduction in the aggregate diffusive capacity of the stomatal population of the plant. As the afternoon advances more and more of the stomates become completely closed, resulting in a progressive diminution in diffusive capacity. This gradual closing of the stomates, resulting in an increasingly larger proportion of them being closed as the day advances, is the principal factor reducing the rate of transpiration during the latter part of the day. The effect of this factor and that of a decreased steepness of the vapor pressure gradient overlap during a large part of the afternoon.

Finally, by late afternoon, complete closure of essentially all of the stomates has occurred. Stomatal transpiration is terminated at that time and during the hours of darkness the rate of water loss from the plant is controlled by factors influencing the rate of cuticular transpiration.

Under environmental conditions deviating very greatly from those which were postulated in the preceding discussion, transpiration periodicity curves may be entirely different from those shown in Fig. 50. Variations in temperature, light intensity, humidity, and soil water supply may all markedly influence both the trend of transpiration periodicity, and the magnitude of the daily water loss.

Low temperatures may result in a complete elimination of stomatal transpiration by inducing stomatal closure.

Low light intensities, such as those existing on cloudy days, are unfavorable to stomatal opening in most species. The stomates seldom open completely under such conditions and the period during which they are open is usually of shorter duration than on clear days. Furthermore, the vapor pressure gradient through the stomates is seldom as steep on such days as on clear, bright days, since leaf temperatures never appreciably exceed atmospheric

temperatures except when exposed to direct sunlight, and atmospheric vapor pressures are usually higher during cloudy days than on clear days at the same season of the year. The magnitude of transpiration under such conditions is usually greatly reduced, and transpiration periodicity curves plotted for plants exposed to such conditions usually present a somewhat foreshortened and greatly flattened appearance.

A deficient soil water supply is most commonly the factor which causes marked departures from the type of transpiration periodicity already considered, especially during the summer months. The effect of a gradual diminution in soil water content upon transpiration periodicity is illustrated graphically in Fig. 51. A reduction in soil water content has two pronounced effects upon the daily march of transpiration. The total daily

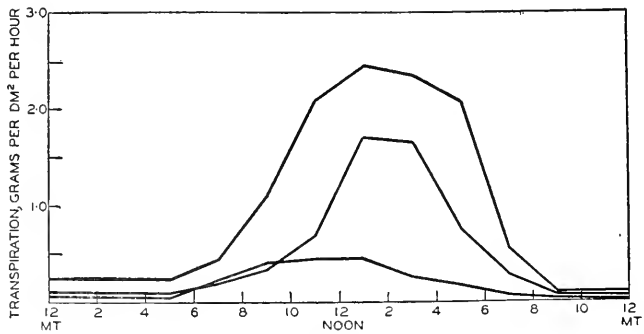

FIG. 51. Daily periodicity of transpiration of bean (*Phaseolus vulgaris*) for three successive days during a period when the soil was gradually becoming dryer. Data of Chung (1935).

magnitude of water loss is decreased, and the peak of the transpiration curve often occurs somewhat earlier in the day than under conditions of abundant soil water supply. Since, even in temperate regions, periods of decreased soil water supply are of common occurrence during the summer months, and in many habitats are the rule rather than the exception, transpiration periodicity curves such as those shown in Fig. 51 are, for many species of plants, much of the time, more nearly representative than the curves shown in Fig. 50.

Internal factors may also be responsible for transpiration periodicities of a different trend from those which have already been discussed. In some species of plants, as has already been described, the stomates remain open to a greater or lesser extent during the hours of darkness. Such plants have higher transpiration rates at night than those in which the stomates are closed. In some species of cacti there is a complete inversion of the usual transpiration

periodicity curve, transpiration rates being regularly greater at night than in the daytime. This appears to be due to complete or nearly complete stomatal closure during the hours of daylight, while the stomates are, as a rule, open at night.

Seasonal Variations in Transpiration Rates.—In temperate regions transpiration occurs predominantly during the warmer months of the year, and especially during those periods of the warm season when the soil water supply is abundant. Among the deciduous group of woody perennials, the branches are defoliated during most of the autumn, all of the winter, and the earlier part of the spring. Although the twigs and branches which remain exposed to the atmosphere are completely encased in corky layers of bark and the buds are enclosed within cutinized bud scales some water loss occurs from such species even during the winter months. Young twigs lose water under such conditions faster than older ones. Winter transpiration rates of deciduous woody plants are always negligible in comparison with summer rates. In evergreen species of either the needle-leaved or broad-leaved type the transpiration rates are usually not appreciably different at most times during the winter from the transpiration rate of deciduous trees at that season (Weaver and Mogensen, 1919). This is probably due principally to the fact that the low temperatures of the winter prevent opening of the stomates although the relatively low leaf water contents characteristic of this season may also be a factor in maintaining the stomates in a closed condition.

Mild periods of any considerable duration often have a detrimental effect upon evergreen species during the winter months. The warm air temperatures induce stomatal opening, and a relatively high rate of transpiration ensues. The soil is apt to be deficient in water at such times, or even if present, that in the surface layers may remain frozen during a period of warm air temperatures. Even if neither of the foregoing conditions exists, the soil temperatures will be low, and this greatly retards the rate of absorption of water. The combined effect of a relatively high transpiration rate and relatively low absorption rate results in a gradual desiccation of the leaves and branches of the plant. This diminution in the water content of the aerial organs during warm periods in winter is more severe in windy weather and is more likely to occur if a sequence of mild days follows immediately after a cold spell. If severe enough this desiccation will result in the death of some of the branches, or in extreme cases of the entire tree or shrub. This is one cause of the phenomenon known as *winter-killing*. Winter-killing due to desiccation of the tissues should not be confused with cold injury due to low temperatures, which is an entirely different phenomenon (Chap. XXXIII).

Discussion Questions

1. Why does the equilibrium vapor pressure of water increase markedly with rise of temperature?
2. What factors determine the vapor pressure of an atmosphere? Why does water-vapor diffuse from a region of greater to a region of lesser vapor pressure?
3. If a pan of water is maintained at a temperature of 30° C. and exposed to the outside atmosphere when would it evaporate most rapidly, in the summer or in the winter?
4. Plot curves which might reasonably be expected to represent the daily variation in the vapor pressure of an atmosphere: (a) out-of-doors on a perfectly clear summer day, (b) out-of-doors on a perfectly clear winter day, (c) in a closed greenhouse during a clear day, (d) out-of-doors on a clear late spring day with a heavy dew in the morning, and (e) out-of-doors during a summer day upon which a heavy thunderstorm occurs in the afternoon.
5. Why is the air of the California and Arizona semi-deserts considered "dry" when it contains approximately the same quantity of water per unit volume as the "moist" air of the Minnesota lake country?
6. Cell A has an osmotic pressure of 30 atmos. and wall pressure of 15 atmos. Cell B has an osmotic pressure of 30 atmos. and a zero wall pressure. Cell C has an osmotic pressure of 30 atmos. and the water within it is under a tension of 15 atmos. From which of these cells will water evaporate most rapidly? least rapidly? Explain.
7. Explain exactly why an increase in leaf temperature usually results in an increase in the rate of transpiration.
8. If half the leaves were removed from a plant what effect would this have on the rate of transpiration from the rest of the leaves? Would the effect be the same under all environmental conditions?
9. Which of the three factors, vapor pressure, sunlight or air temperature do you think usually has the greatest influence on transpiration rates under field conditions? Which second? Why? What fourth environmental factor is often of greater importance than any of these three?
10. Under what conditions would transpiration be approximately proportional to the vapor pressure deficit of the atmosphere? When not?
11. Assuming 100 per cent relative humidity in the intercellular spaces, open stomates, and sufficient air movement to prevent appreciable accumulation of water-vapor around the leaves, which plant will lose water-vapor more rapidly—one in an environment of 80 per cent relative humidity and 40° C. temperature, or a similar one in an environment of 80 per cent relative humidity and 20° C. temperature? Explain.
12. Making the same assumptions as in question 11, which plant will transpire more rapidly—one with a leaf temperature of 35° C. in an atmosphere of 30° and relative humidity of 70 per cent or one with a leaf temperature of 30° C. in an atmosphere of 30° C. with a relative humidity of 60 per cent. Explain.
13. Making the same assumptions as in question 11, which would have the greater effect on the rate of transpiration—an increase in leaf temperature from 30 to 35° C. or a decrease in atmospheric relative humidity from 80 to 60 per cent, air temperature remaining at 30° C.? Explain.
14. The transpiration of a potted tobacco plant was measured hourly during a

24 hour period by the hygrometric paper method and by weighing. The transpiration of a similar tobacco shoot from which the roots had been removed was measured during the same period with a potometer. Plot curves which might reasonably be expected to represent the results of each of these series of determinations on a "standard day" and explain any differences in the shape of the curves.

15. The vapor pressure of the atmosphere is often relatively constant upon clear summer days. Measurements of the vapor pressure of the air close to plants often show appreciable variations from hour to hour during the day. How may these apparently contradictory facts be explained?
16. A plant is growing under conditions of constant light, temperature, and humidity in a current of air from a fan. The fan is turned off for a few minutes, and the rate of transpiration decreases. If the fan is now started again the rate of transpiration is found to rise to a higher value than just before the fan was turned off. Explain.

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CHAPTER XV

THE MOVEMENT OF WATER THROUGH THE PLANT

Most terrestrial plants obtain the water which is necessary for their existence from the soil. An overwhelmingly large proportion of the water which is absorbed by the roots of land plants is lost in the process of transpiration. Smaller quantities are utilized in growth and in photosynthesis, and in some species limited amounts of water may be lost by guttation. Water must therefore move through the intervening tissues and organs from the absorbing regions of the root to the tissues in which it is utilized, or from which it passes out of the plant. The process whereby water moves through the plant is often termed the *conduction, transport*, or *translocation* of water.

In herbaceous species and many shrubby plants the distance through which water moves in passing from the root tips to the leaves is usually not more than a few feet. Even in such plants appearances are sometimes deceptive, as some herbaceous and shrubby species such as alfalfa may have such deep root systems that some of the absorbed water often ascends for distances as great as twenty or more feet before it reaches the level of the soil surface. It is in trees, however, in which the most striking illustrations of the upward movement of water occur. The tallest tree of which we have an authentic record is a specimen of the Coast Redwood (*Sequoia sempervirens*) which has attained a height of 364 feet (Tiemann, 1935). Many other individuals of this species and of several others, including the Big Trees (*Sequoia gigantea*) of California, the Douglas Firs (*Pseudotsuga mucronata*) of the Pacific Northwest, and the Blue Gums (*Eucalyptus*) of Australia exceed 300 feet in height. Trees ranging from 100 to 200 feet in height were of common occurrence in the virgin forests of eastern North America. Since the root systems of trees always penetrate at least a few feet into the ground, the actual vertical distance through which at least a part of the water absorbed is elevated is always more than the height of the tree. In trees, therefore, water must ascend to heights ranging up to nearly 400 feet above the level of water absorption.

The mechanism by which this feat is accomplished in tall trees has been

the subject of much experimentation, and even more speculation. The ensuing discussion of the "ascent of sap" through plants will be presented largely in terms of its movement through trees, principally because much of the experimental work on this problem has been performed on woody species. Any explanation of this phenomenon which can be shown to be adequate for tall trees should also prove satisfactory for vascular species of lesser stature.

The Path of Water Through the Plant.—Water enters the plant through the epidermal cells and root hairs at or near the tips of the roots and crosses the cortex, endodermis, and a part of the pericycle before it finally

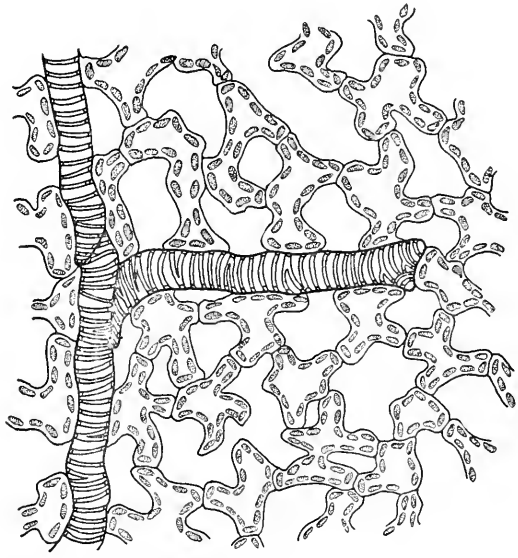

FIG. 52. Terminus of xylem elements in mesophyll. Redrawn from Eames and MacDaniels (1925).

enters the lumina of the vessels or tracheids of the root xylem (Fig. 70). At this point its upward movement begins. The xylem tissue is continuous from just back of the root tips, through the roots, into and through the stems, the petioles of leaves, and ultimately, usually only after much branching, terminates in the mesophyll of the leaf (Fig. 52). The xylem tissue through which the water moves is thus a continuous unit system within the body of the plant. Along most of its course water moves *en masse* through the vessels or tracheids of the xylem. At the termination of the xylem ducts in the leaves the water passes into the adjacent mesophyll cells. In the mesophyll of the leaves it moves from cell to cell, eventually most of it being lost from the cells by

evaporation into the intercellular spaces. The movement of water through the cells of the root and leaf mesophyll must be regarded as integral parts of the process of translocation of water.

While the great bulk of the water which passes through the plant follows the route just described, and is lost in the transpirational process, small quantities escape this fate. All along the route of its movement small amounts of water pass into adjacent living cells and are utilized in cell enlargement, especially in the cambium layer. Actively growing stem or root tips, fruits, etc. also utilize considerable quantities of water principally in the enlargement phase of growth, while chlorenchyma cells utilize water in the photosynthetic process. In most species, however, not more than 1 or 2 per cent of the water which enters a plant is utilized in growth and metabolic processes, the remainder being lost from the plant in transpiration.

That the xylem is the water-conducting tissue of plants has been recognized at least since the time of the girdling experiments of Malpighi in 1671. Girdling (or "ringing") a stem, so that all of the tissues external to the xylem are removed, does not prevent movement of water to organs attached to that stem above the ring. On the contrary cutting through the xylem tissue of a stem results in almost immediate wilting of leaves attached to the stem above the ring.

Anatomy of Stems.—Since the mechanism of the movement of water can scarcely be understood intelligently without some knowledge of the anatomy of the tissues through which it moves, a brief review of stem structure is desirable before proceeding further with a discussion of this process. Stems vary greatly in their structure, every species possessing some anatomical features which are peculiar to itself. Nevertheless certain general patterns of tissue arrangement have been found to prevail, and the stem structure of most species will approximate one or another of these general arrangements.

The stem anatomy of two representative species is shown in Fig. 53 and Fig. 54, which are self-explanatory. The corn stem represents an herbaceous monocot type of structure while that of the tulip poplar is typical of woody dicot stems.

The structure of woody stems cannot properly be appreciated merely from a consideration of one year old stems. The *primary tissues* of such stems develop from tissues formed at the apical growing tip during growth of the stem in length. Nearly all perennial stems also grow in diameter as a result of the development of *secondary tissues* from the cambium. By successive stages of division, enlargement, and differentiation of cambium cells additional layers of xylem (*secondary xylem*) are laid down on the inner face of the cambium, and new layers of phloem tissue (*secondary phloem*) on its outer

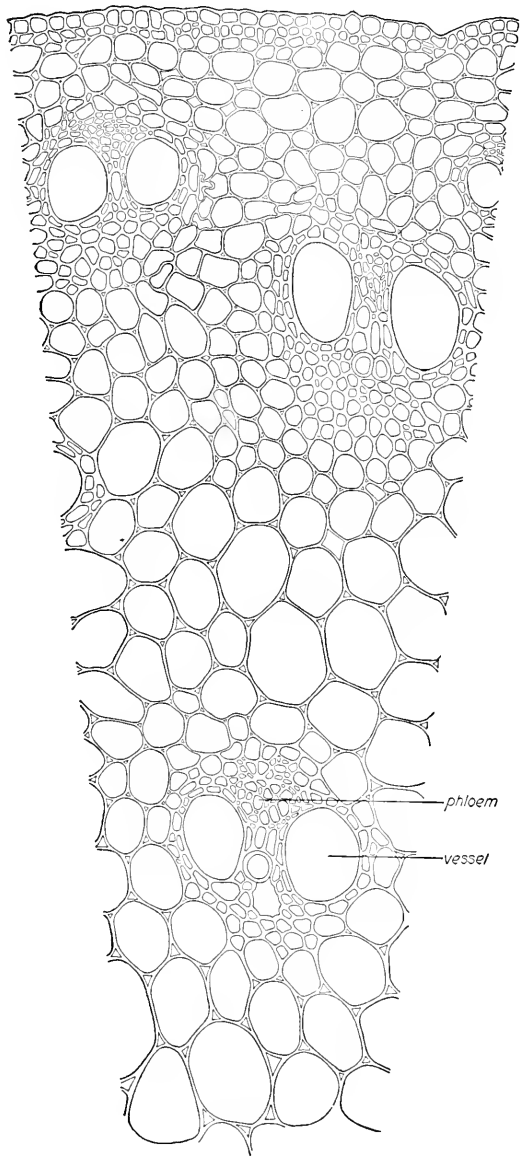

FIG. 53. Segment of young corn stem as seen in cross section. Three vascular bundles and parts of two others are shown. The large-celled tissue between the bundles is sometimes classed as pith; sometimes as parenchyma.

face (Fig. 55). Secondary growth of woody stems is initiated during the first season of their development, and continues during each growing season

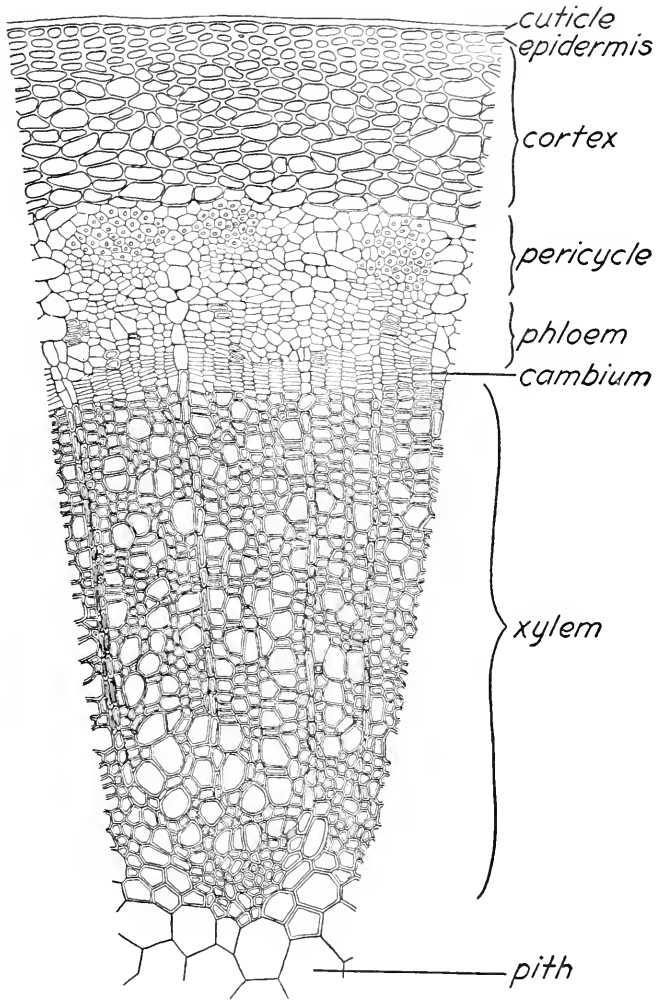

FIG. 54. Segment of young stem of tulip poplar (*Liriodendron tulipifera*) as seen in cross section.

thereafter. Hence after a few years the great bulk of any woody stem is composed of secondary tissues. The apical and diameter growth of woody

stems and the relation of these processes to the production of the primary and secondary tissues of the stem is considered more in detail in Chap. XXXI.

Secondary xylem and secondary phloem also develop from the cambium in most herbaceous dicot stems but in such species the cambium in any one stem is never active for more than one growing season.

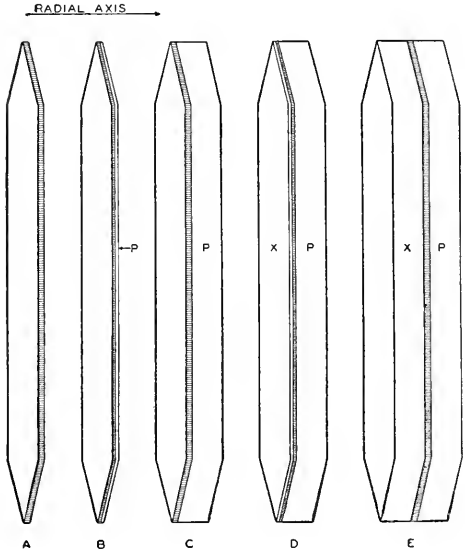

FIG. 55. Diagram illustrating formation of phloem and xylem elements from the cambium. (*A*) a cambium initial, (*B*) division of cambium cell, (*C*) outer cell resulting from division becomes a phloem element, (*D*) another division of the cambium cell, (*E*) outer cell resulting from second division becomes a xylem element. Actually several xylem or several phloem elements are often formed successively. Upon maturation most of the xylem and phloem elements acquire sizes and shapes which are very different from those of the cambium initials from which they originate.

The spring formed xylem tissue, as viewed in cross section, is usually distinctly different in aspect from that formed later in the season. In many angiosperms the "spring" wood contains more and larger vessels and the cell walls are generally thinner than in the subsequently produced "summer" wood. In the conifers the spring formed tracheids are thinner-walled and of larger cross-sectional diameter than those formed later in the growing season. The transition from spring to summer wood is often a very gradual one. On the other hand the more open xylem tissues formed each spring abut directly upon the denser tissues which were produced during the preceding summer, thus giving rise to an abrupt line of demarcation between the zones of xylem formed in any two successive seasons. The result of this growth behavior is that a cross section of the trunk or a branch of any tree appears as a system of concentric layers, the so-called *annual rings,* each representing an annual increment of growth. In rare cases no annual rings nor more than one annual ring may be produced in a season, but usually each ring represents the xylem produced by the activity of the cambium during one season.

In many woody species as the xylem tissues increase in age important

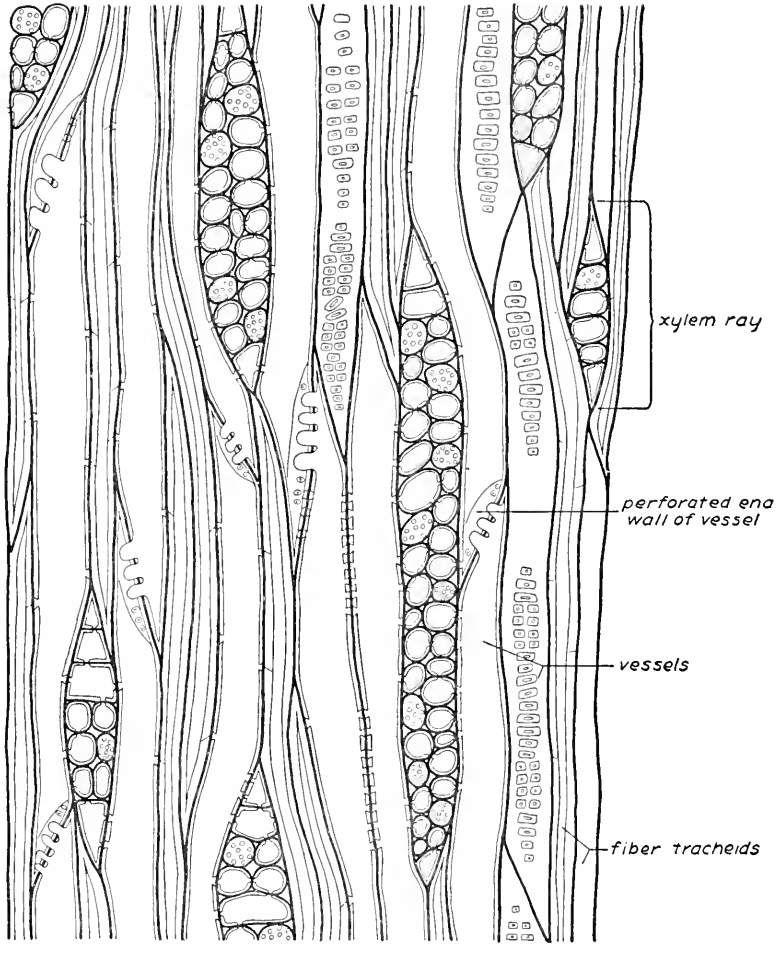

FIG. 56. Tangential section of a small portion of the wood of a tulip tree (*Liriodendron tulipifera*). This is a plane section except that the end walls of the vessels have been shown in perspective in order to indicate their structure clearly. Most of the vessels and fiber tracheids show in sectional view, but a few show as the surface view of the tangential wall. Surface and sectional views of bordered pits show in the vessel walls. Simple pits show in the end walls of ray cells and between ray cells. Half-bordered pits show between vessels and ray cells. Adapted from a drawing by L. G. Livingston.

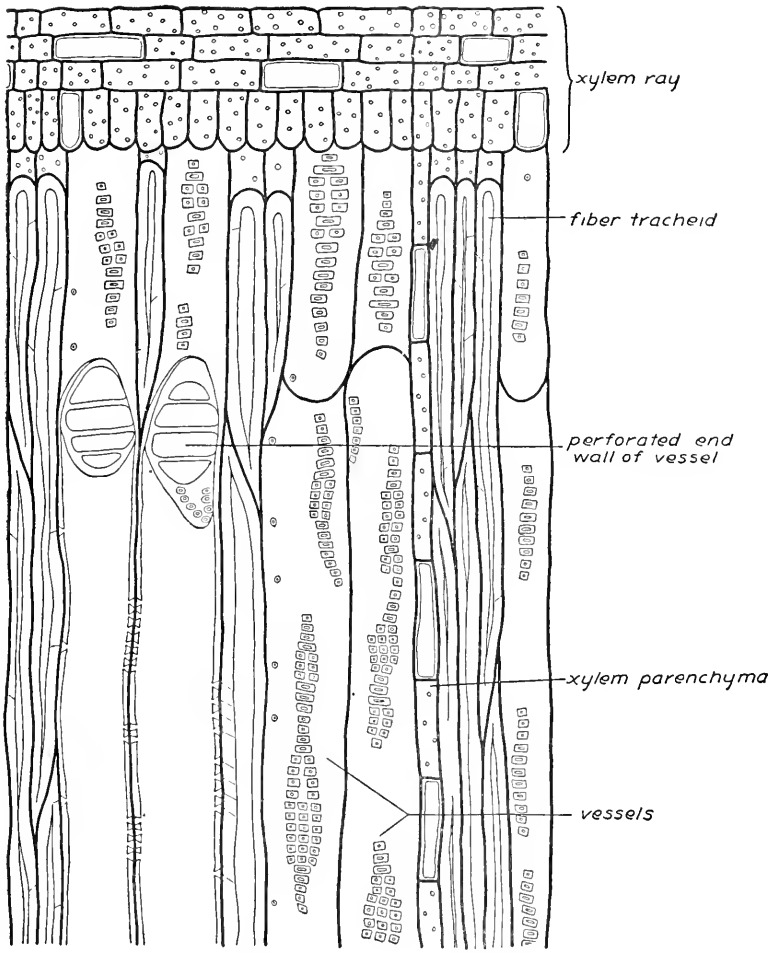

FIG. 57. Radial section of a small portion of the wood of a tulip tree (*Liriodendron tulipifera*). Most of the vessels show as a surface view of the walls; two are in sectional view. Surface and sectional views of bordered pits show in vessel walls. Simple pits show in surface view in the walls of the xylem ray and xylem parenchyma cells. Adapted from a drawing by L. G. Livingston.

changes occur in the color, composition, and structure of the various elements, resulting in the conversion of *sapwood* into *heartwood.* As sapwood ripens into heartwood the walls of any remaining living cells of the xylem become increasingly lignified, death of these cells soon following. The water content of the tissues is generally reduced and such compounds as oils, resins, gums, and tannins accumulate in the cells or cell walls. The darker coloration of the heartwood of most species as compared to the sapwood is due to such accumulations.

In mature trees the heartwood becomes merely a central supporting column surrounded by a cylinder of sapwood which varies in thickness from a few to many annual layers, depending upon the species and the environmental conditions under which the tree is growing. In some species (apple, elm) the heartwood remains virtually saturated

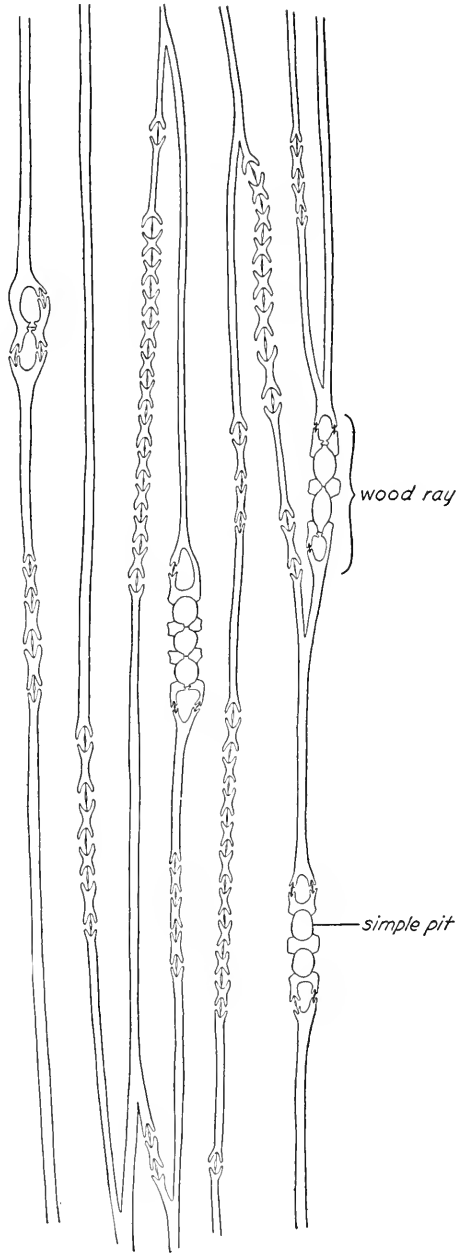

FIG. 58. Tangential section of a small portion of the wood of white pine (*Pinus strobus*). The vertically oriented elongated elements are tracheids. Bordered pits show in sectional view in the tracheid and marginal ray cells. Simple pits show in sectional view in other ray cells. Half-bordered pits show between marginal ray cells and other ray cells.

with water, while in others (ash) it becomes relatively dry. The water in the heartwood of such species as apple and elm appears to be largely static and is not directly involved in translocation.

Coincident with the development of secondary xylem, secondary phloem tissues also develop from the cambium (Chap. XXVIII). Cork cambiums are also initiated in the bark which produce cork layers. Profound modifications therefore occur in the outer tissues as well as in the xylem of woody stems as they grow older.

A somewhat more detailed concept of the structure of the cells and elements of the stems of angiosperms through which the movement of water occurs is presented in Fig. 56 and Fig. 57, which represent longitudinal tangential and radial sections from the xylem of a tulip tree which may be taken as representative for this group of plants. Fig. 58 and Fig. 59 illustrate in a similar way the structure of the xylem tissues of the white pine—a representative gymnosperm. The following discussion merely amplifies somewhat the facts depicted graphically in these two figures.

The xylem tissues of the wood of angiosperms are composed of vessels, tracheids, fibers of several types, wood parenchyma, and xylem ray cells. There is a tremendous variability in the proportional distribution and arrangement of these tissues according to species.

The most characteristic elements of the xylem tissue of angiosperms are the vessels. These are, in general, more or less tubular structures which may extend through many feet of the xylem. In some species cross walls, usually perforated, are of frequent occurrence in vessels, in others such cross walls are infrequent or lacking. In diameter they may range in trees from about 20 μ to about 400 μ. In vines they may be as much as 700 μ in diameter. The vessels branch extensively in certain regions of the plant, especially at nodes, within the leaf lamina, and at the points in the root systems where root branching occurs.

The vessels of the *protoxylem* (the first cells of the xylem to mature during the ontogeny of a growing stem or root tip) have cellulose walls which are reenforced by distinctive lignified thickenings which appear as rings, spirals, or other characteristic patterns. The vessels that develop later in the ontogeny of any growing tip have lignified, usually pitted walls which lack any of the thickenings which distinguish the walls of the vessels of the protoxylem.

Pits normally occur in all parts of vessel walls which are contiguous with other vessels or cells. These pits consist essentially of thin areas in the walls. The walls of the vast majority of plant cells are pitted. The architecturally distinct type known as the *bordered pit* is characteristic of most

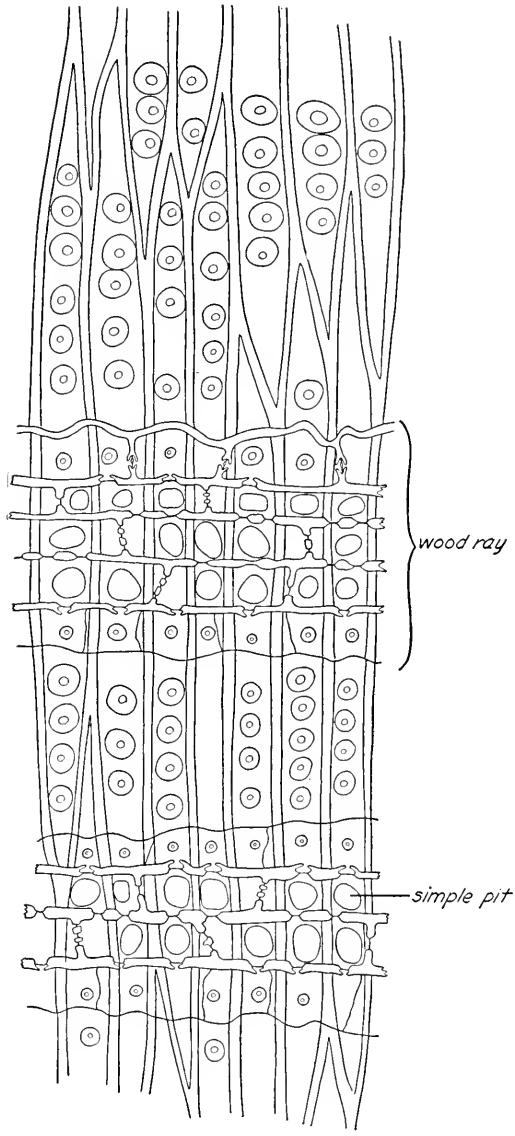

FIG. 59. Radial section of a small portion of the wood of white pine (*Pinus strobus*). The vertically oriented elongated elements are tracheids. Bordered pits show in face view in the tracheids; in sectional view in marginal ray cells. Simple pits show in surface and sectional views in the ray cells. Half-bordered pits show between marginal ray cells and other ray cells. Three rows of marginal ray cells show in outline only.

water-conducting cells and vessels (Fig. 60, *A*). *Simple pits,* on the other hand, are restricted almost exclusively to the walls of living cells (Fig. 60, *B*).

The stages in the development of a vessel, which is a more complex phenomenon than the formation of the other elements of the wood, are indicated in Fig. 61. The original cell resulting from division of a cambium cell increases rapidly in diameter, simultaneously developing a prominent vacuole. Lignification of the lateral walls of the cell takes place at about the same time that dissolution of the end walls of the *vessel segment* occurs. Disintegration of the protoplasm also occurs at this stage in vessel development. The result of this series of processes is the formation of a typical tubular, non-living *vessel* by the coalescence of a number of vessel segments each of which

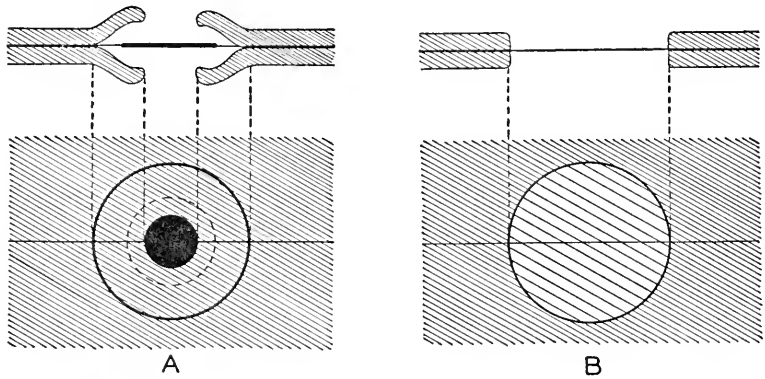

FIG. 60. Diagrammatic representation of (*A*) bordered pit, sectional (above) and face views, (*B*) simple pit, sectional (above) and face views. Redrawn from Eames and MacDaniels (1925).

has been differentiated from a single cell originating from a division of a cambium cell. In many species some of the cross walls between vessel segments fail to disintegrate. Such cross walls are almost invariably perforated by one or more openings (Fig. 57). The distance between the residual cross walls in xylem ducts varies greatly from species to species and often from vessel to vessel in the same individual plant. There is some evidence that the distance between the cross walls in the vessels of ring porous species is usually greater than in diffuse porous species (Handley, 1936). According to Priestley (1935), in some species of trees at least, disintegration of the end walls occurs almost simultaneously between all of the newly differentiated vessel segments which are present as a continuous chain of cells from the top to the bottom of the tree.

Tracheids are found in wood of many but not all species of angiosperms. They are typically more or less spindle-shaped cells with thick walls which are almost always lignified. As viewed in cross section they are usually angular. Mature tracheids contain no protoplasm and hence are non-living like the xylem vessels. The largest tracheids are about 5 mm. in length, and about 30 μ in diameter, although most of them are smaller. The walls of tracheids are pitted like those of vessels. Tracheids are water-conducting cells but in most angiosperms they are relatively of much less importance as channels through which water moves than the vessels.

Tracheids are developed from the cambium by a process essentially similar to that by which vessel segments are formed, except that the size and shape of

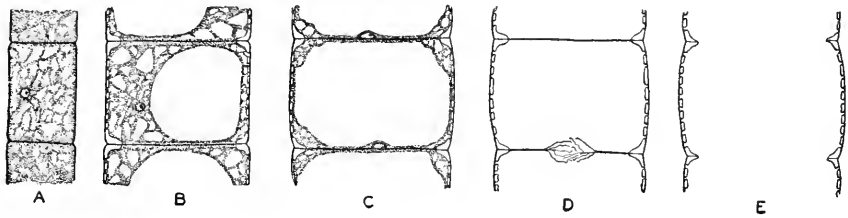

FIG. 61. Stages in the development of a vessel. (*A*) enlarged cambial cell, (*B*) cell still further enlarged with secondary wall developed and pits formed, (*C*) cytoplasm now only adjacent to walls, nucleus against transverse wall, (*D*) cytoplasm has disappeared, transverse walls disintegrating, (*E*) transverse walls have disappeared; transformed cell now part of vessel. Redrawn from Eames and MacDaniels (1925).

the resulting cells is different, and that there is no coalescence of the individual elements such as occurs in the formation of vessels.

The xylem of the angiosperms also contains fibers of various types, which in general are similar to the tracheids in structure except that usually they have thicker walls, smaller lumina, and fewer and smaller pits. Fiber cells are non-living and their walls are lignified. Because of their thick walls and small lumina the fibers do not play an important part in the movement of water through stems.

The xylem tissue of practically all angiosperms also contains wood parenchyma cells which, unlike the elements already described, remain alive for some time after their differentiation. Usually death of the wood parenchyma cells does not occur until the wood of which they are a part is converted into heartwood. Wood parenchyma cells are generally somewhat elongate, and roughly four-angled in cross section. They occur in the xylem as vertical series of cells placed end to end. The strands of wood parenchyma thus

produced often extend for a long distance in a vertical direction through the wood. The distribution of the wood parenchyma strands throughout an annual ring of xylem tissue varies with the species. In some they are more or less scattered through the xylem, in others they occur only in the last layer or two of cells which are produced in the summer wood—in other words at the termination of the season's growth—and in others only in contact with the vessels, or in contact with other wood parenchyma cells which are themselves in contact with the vessels.

All of the xylem elements previously described are oriented with their long axes in a vertical direction. In addition to this vertical system there is also present in the xylem a transverse, radiating system in which the long axis of the cells is at right angles to the long axis of the stem. These transversely oriented tissue units are known as *vascular rays*. In the stems of most species they are continuous from the outer extremity of the phloem through the cambium into the xylem, which they penetrate to a greater or lesser distance. The portion of the vascular ray found in the xylem is termed the *xylem ray;* that found in the phloem the *phloem ray*. Xylem rays may vary from one to many cells in thickness and likewise in height, certain types usually being characteristic of any one species. The cells of the xylem rays, like those of the wood parenchyma, usually remain alive until the woody tissue is converted into heartwood. The cells of the xylem ray are typically elongate and more or less angular in cross section. The xylem rays probably serve as routes along which lateral movement of water occurs from the xylem to the cambium and phloem, and along which translocation of soluble foods takes place from the phloem to the living cells of the xylem.

The living ray cells are in contact at various points with the strands of living wood parenchyma cells. The vertically oriented wood parenchyma strands and transversely oriented xylem ray strands thus form a unit system of living cells within the woody cylinder. Hence there is present a continuous intermeshing network of living cells throughout the greater mass of nonliving vessels, tracheids, and fibers in the younger portions of any woody angiosperm stem. There are probably few if any of the conducting elements—vessels and tracheids—which are not in contact at one or more points with this continuous system of living cells.

The wood of the gymnosperms is simpler in its structure than that of the angiosperms, this group of woody species also showing, in general, a greater uniformity in stem structure than the latter group. The only cell types universally present in the wood of coniferous trees are the tracheids and wood ray cells. Wood parenchyma cells are also present in the wood of most species of conifers, while in many species tracheid-like fiber cells are also

present. The most important distinction between the wood of conifers and that of angiosperms is the total absence of vessels in the former.

The tracheids are the distinctive element in conifer wood, constituting as they do the great bulk of all the woody tissues present in such species. In conifers the tracheids form a densely packed type of woody tissue, composed of interlocking cells. Vertically contiguous tracheids always overlap along their tapering portions. Movement of water and solutes from one tracheid to another is facilitated by means of the bordered pits in the adjacent walls. Because of the numerous cross walls in a xylem tissue composed almost entirely of tracheids, water encounters a greater resistance in moving through such tissues than in traversing woody tissues which contain vessels. Nevertheless it is interesting to note that the tallest trees in the world are conifers in which all movement of water occurs through tracheids.

Theories of the Mechanism of the Movement of Water through Plants.—A number of different theories of the mechanism by which the ascent of water is brought about in plants have been suggested, and it is probable that more than one mechanism is involved in this process. The present state of our knowledge justifies a discussion of only three possible mechanisms: (1) that ascent of sap is caused by the "vital" activities of living cells, principally those in the stem, (2) that this process occurs according to a mechanism described in the "cohesion of water" theory, and (3) that upward movement of water occurs as a result of "root pressures." In passing, it should be noted that neither atmospheric pressure nor capillarity can account for the rise of sap in plants, although both of these processes are recurringly suggested as being involved in this process. Atmospheric pressure could never account for a rise of more than about 10 meters, and most mature trees attain a greater height than this. The capillary rise of water in tubes 10μ in diameter is only about 3 meters, and few if any of the conducting elements have as small a diameter as this.

The mechanism of the movement of water through the plant is inextricably bound up with the mechanism of the absorption of water, so the following discussion will necessarily treat to some extent of both of these processes.

"Vital" Theories.—While the vessels and tracheids through which longitudinal transit of water occurs are non-living, they are always in more or less intimate contact with living cells. Suggestions have therefore been made from time to time that the motive power causing movement of water is furnished by the living cells of the stem. The most recent advocate of such a theory is Bose (1923).

As an example of the "vital" theories of the ascent of sap we may consider the proposals advanced by Godlewski in 1884. He considered that

conduction of water occurred as a result of periodic changes in the osmotic pressure of the living cells present in the xylem (especially the wood ray cells). Upon increase in their osmotic pressure water was supposed to move into the cells from bordering xylem elements. Upon decrease in osmotic pressure water was supposed to move back into a xylem duct. In the xylem duct it was supposed that water would move upward until it came in contact with another living ray cell because of a lower air pressure in the upper than in the lower part of the vessels. The ascent of water in stems was thus supposed to be brought about by a number of repetitions of this same process. There is little or no direct evidence in support of this or any of a number of more or less similar theories which have been suggested.

The experiments of Strasburger (1893) demonstrated quite clearly that the primary mechanism of the rise of sap in trees operates independently of the living cells of the stem. He performed many experiments upon the movement of water through woody stems in which the living cells had been killed by one method or another. In one experiment, for example, he used a 75-year-old oak tree about 22 meters in height. This was sawed off close to the ground and the cut end transferred to a solution of picric acid, which is toxic to living cells. The picric acid solution slowly moved up the stem. Fuchsin, added to the liquid in which the base of the tree was immersed 3 days after the picric acid, also ascended to the top of the tree through tissues in which the living cells had been killed by the picric acid. Water also continued to ascend through similar stems after they had been completely killed by exposure to a temperature of 90° C.

Similar experiments have been performed by later investigators, especially Roshardt (1910), Overton (1911), and MacDougal, *et al.* (1929). All of these investigators have confirmed Strasburger's results that water would continue to ascend for some time through stems, segments of which had been exposed to one treatment or another which would kill all living cells present.

In all experiments of the type just described, it has been observed that the leaves at the top of a stem, part or all of which has been killed, sooner or later wilt and wither, although this effect is by no means an immediate one, often appearing only after several days. Proponents of the vital theories have accepted this as evidence that the living cells of a stem are essential for the passage of water through it. Dixon (1914) however, considers that this delayed lethal effect on the leaves is due to either or both of two causes. Killing of the stem tissues often causes the formation of substances which plug up the vessels or tracheids, thus impeding the upward movement of water. Furthermore, death of the cells in the treated regions causes the release into the conducting channels of toxic compounds which, when transported to the

leaves, cause death of the leaf cells. There is still some doubt, however, whether all of the retardation in rate of translocation as a result of such experiments can be explained in these two ways. The possibility remains open that the living cells of the xylem may in some way be necessary to the maintenance of the water-conducting capacity of the xylem, or even that they may contribute in some way to the lifting of water through stems (Ursprung, 1912).

In general, the evidence appears to be quite conclusive that the living cells of the stem do not furnish the principal mechanism which causes the upward transport of water in stems. On the contrary, as shown in the subsequent discussion, the living leaves or stem tips at the top of the plant appear to be absolutely necessary for the ascent of water through stems.

"Root Pressure."—That a slow exudation of sap often occurs from the cut surface of a stem is well known. Such sap exudations are often ascribed to the development of a pressure in the dilute sap of the xylem vessels resulting from the operation of some as yet not fully understood mechanism in the root cells. Hence the name "root pressure." It is generally considered that such pressures may occur in intact plants as well as in those which have been cut into.

It is probable that not all of the phenomena which have been classed under the name of "root pressure" are due to the operation of the same physiological mechanism. Some sap exudations seem to come from the cambium or phloem rather than from the xylem. It seems quite certain that some of the sap exudations from the xylem occur from the living wood parenchyma or wood ray cells rather than from the vessels, or at least that the exudation is greatly influenced by the activity of such cells. Hence the living cells of the stem as well as those of the root are probably involved in many "root pressure" phenomena. James and Baker (1933) go so far as to regard the subjection of the sap in vessels to a pressure as an exceptional occurrence in plants, but this seems to be an extreme point of view, and will not be followed in this discussion. Because of the unsettled state of our knowledge regarding this phenomenon it will be necessary to use the term "root pressure" in a very uncritical sense.

The magnitude of "root pressures" is commonly determined by attaching a manometer to the cut surface of the plant from which the bleeding is occurring. With only a few rare exceptions the exudation pressures which have been recorded for plants do not exceed 2 atmos., and most of them are less than this. According to White (1938) individual excised roots of tomato plants exude sap from their basal ends with a pressure of more than 6 atmos. It has not yet been shown, however, that such pressures operate for any con-

siderable distance through plants. In general there is no correlation between the pressure with which the sap is exuded from cut stems and the volume of sap flow. In some species relatively large volumes of sap may be exuded under a relatively low pressure, in others exactly the opposite relation may exist.

Some of the woody plants in which bleeding from cut surfaces occurs, commonly ascribed in part or entirely to root pressure, are the sugar maple, box elder, dogwood, hornbeam (*Ostrya*), sycamore, birches, currant, and grape. Such exudation of sap under pressure usually occurs only during the seasons of the year when the plant bears no foliage, and most commonly in the early spring months. The popular concept of a "rise of sap" in woody plants in the spring is largely based on the observation of such phenomena. Exudation of sap under pressure can also be demonstrated in many species of herbaceous plants especially if potted plants are used and the soil is flooded with water before the demonstration is attempted.

The sap which moves through woody plants in the spring and, less abundantly, in the autumn under the influence of "root pressures" contains appreciable concentrations of carbohydrates as well as traces of mineral salts. In contrast with this is the condition of the xylem sap in the summer at which season it contains traces of mineral salts, but practically no soluble carbohydrates (Fig. 107, Fig. 109).

It has sometimes been claimed that "root pressures" are adequate to account for the rise of water in herbaceous plants and in low shrubs or trees, and that they may even play a very considerable part in the ascent of sap in taller trees. While it is undoubtedly true that "root pressure" does, in some species of plants, under certain conditions, account for the movement of some water in an upward direction through plants, this process is inadequate to account for the translocation of any considerable amount of water through plants. In the first place there are many species in which this phenomenon has never been observed. In the second place, the magnitude of the pressure developed is seldom sufficient to force water to the top of any except relatively low-growing species of plants. Neither is the rate of flow, as it occurs in most species, anywhere near rapid enough to compensate for the known rates of transpiration. Finally, and this is perhaps the most fundamental objection to the idea that "root pressures" play a very prominent part in the ascent of sap in plants; they are usually negligible, in temperate regions at least, during the summer period when transpiration is most rapid. During periods of rapid transpiration, the cut surfaces of plants not only fail to exude sap, but will usually absorb water if it is supplied at the cut surface.

The trunks of many species of deciduous trees exhibit a marked seasonal

variation in water content (Fig. 62). A minimum water content is attained during middle or late summer and the trunks gradually fill up during the fall and spring. Often there is a temporary shrinkage in their water content during the winter months but the main period of diminishing trunk water content occurs in the spring and summer. Some authorities even speak of a seasonal rise and fall in the "water table" of a tree trunk, and consider that intercellular spaces as well as cells become occupied with water during this process (Priestley, 1930). "Root pressure" undoubtedly accounts at least in part for such seasonal replenishments of the store of water in tree trunks.

FIG. 62. Seasonal variation in the water content of the trunks of the trembling aspen (*Populus tremuloides*). Data of Gibbs (1935.)

The living cells of the xylem probably play an active part in this process. It is possible that there may be slowly ascending streams of water in the living cells of the xylem which operate entirely independently of the water columns in the vessels or tracheids. Hence the same portions of the xylem which at times contain gases may at other seasons be occupied by water. The trunks of many conifers show no marked seasonal variations in water content; this is possibly correlated with the lack of appreciable root pressures in such species.

The Cohesion of Water Theory.—The leading advocate of this theory has been Dixon (1914, 1924) who has performed much of the experimental work upon which it is based. A number of other workers, notably Askenasy (1897) and Renner (1911, 1915), have also contributed important theoretical and experimental evidence in support of this theory.

Molecules of water, although ceaselessly in motion, are also attracted to each other by strong cohesive forces. In masses of liquid water the existence of such cohesive forces is not obvious, but when water is confined in long

tubes of small diameter the existence of attractive forces between water molecules can often be demonstrated. If the water at the top of such a tube be subjected to a "pull" the resulting stress will be transmitted all along the column of water, due to the mutual attraction between the molecules. The water conducting system of plants constitutes just such a system, enclosing continuous thread-like columns of water which extend from the top to the bottom of the plant. A stress applied at any point to this system will be propagated to all its parts. The application of such a stress stretches the water into taut threads, throwing it into a condition of tension, which is the equivalent of "negative pressure."

According to this theory forces develop in the upper parts of plants, and especially in the leaves, which cause the rise of water through the plant. As evaporation proceeds from the walls of the mesophyll cells into the intercellular spaces the diffusion pressure deficit of the mesophyll cell walls increases. Water therefore moves into the walls from the adjacent protoplasm, this resulting in turn in the movement of water from the cell sap into the protoplasmic layer. The resulting increase in the diffusion pressure deficit of the cell sap is in turn propagated to the protoplasm and the cell sap in all parts of the cell. The osmotic pressures of the leaf cells are generally high enough that diffusion pressure deficits of sufficient magnitude to account for movement of water to the top of the plant can develop in them without a complete loss of turgor by the leaf cells. If the cell is in direct contact with one of the branches of the xylem ducts which ramify throughout the lamina of the leaf, water will move from the vessel or tracheid into the cell; this results in the development of tension in the water column terminating in this xylem element. The diffusion pressure deficit of the water in the conducting elements will be increased by the amount of this tension. Water under a tension ("negative pressure") of 10 atmos., for example, has a diffusion pressure just 10 atmos. less than that of pure water at the same temperature which is not under tension (Chap. XI). In other words its diffusion pressure deficit is 10 atmos. If the cell from which evaporation is occurring is not in direct contact with a xylem element a gradient of diffusion pressure deficits, gradually increasing in magnitude from cell to cell in the direction in which the water is moving, is established between the xylem element and the cell from which evaporation is occurring.

The tension developed in the conducting elements is transmitted along their entire length to their lower termination just back of the root tips and probably, very often at least, across the root tissues as well. The magnitude of the tension which develops in the xylem conduits is increased by conditions

which favor a rate of water loss from the leaves considerably in excess of the rate at which water enters the roots from the soil.

The movement of water across the cells of the root from the soil into the lower ends of the water-conducting elements must be considered as an integral part of the translocational process. At their lower terminations the xylem vessels or tracheids are in contact with the pericycle, or more rarely, directly with the endodermal cells of the root (Fig. 70). Water moves from the adjacent root cells into the conducting elements because the tension (diffusion pressure deficit) developed in these elements exceeds the diffusion pressure deficit of the cells of the root. When the tension in the water columns is relatively low a gradient of diffusion pressure deficits will be established across the root cells similar to those which are established in the leaf mesophyll cells when water is moving through them. The diffusion pressure deficits will increase from cell to cell along this gradient in the direction in which water is moving, *i.e.* from the periphery of the root towards the xylem.

Since, however, the osmotic pressures of root cells are usually lower than those of the mesophyll cells (Hannig, 1912), it is probable that the tension developed in the water columns often exceeds the highest osmotic pressures of any of the root cells through which water passes on its way from the soil to the xylem. Under such conditions the volume of water in the root cells may continue to diminish even after their turgor has been reduced to zero. As a result the walls of the cell are pulled inwards due to the adhesion between them and the contracting mass of water. The counter pull exerted by the elastic walls of the cell will stretch the encompassed mass of water and throw it into a state of tension (Chap. XI). Whenever a tension of sufficient magnitude has been generated in the conducting elements, therefore, the water in the root cells may also pass into a state of tension (Livingston, 1927). Even under such conditions a gradient of diffusion pressure deficits would be established across the root, but the mechanism responsible for the development of this gradient would be more complicated than were no tension generated in the cells. When the water in a cell is under tension its diffusion pressure deficit is equal to its osmotic pressure plus the tension to which the water is subjected (Chap. XI).

Regardless of the exact mechanism involved, the essential fact is that the development of tensions in the water columns induces the establishment of a gradient of diffusion pressure deficits, increasing consistently from cell to cell across the root from its peripheral cell layer to the conducting elements, and water will move along this gradient from the epidermal layer to the xylem tissue. Water enters the peripheral cells of a root whenever the dif-

fusion pressure deficit of water in the soil is less than that of the water in the epidermal cells and root hairs in the absorbing zone of the root (Chap. XVII).

In most trees practically all upward movement of water occurs in the vessels or tracheids of a few of the outermost annual rings of the sapwood and in some species, especially those with ring-porous wood (Huber, 1935) is almost entirely confined to the outermost ring. As is well known, in some species of trees (beech, sycamore, etc.) the heartwood of the trunk may disappear completely by decay. The fact that such hollow trees continue to live and thrive is conclusive evidence that the heartwood plays no essential role in the upward movement of water in such species.

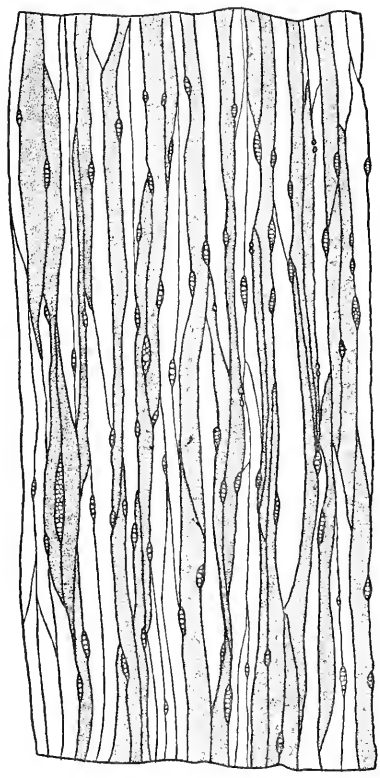

FIG. 63. Continuity of water columns through the wood of a conifer. Redrawn from Dixon (1914).

The water-conductive system of plants should be regarded as a unit system; the individual threads or columns of water not operating separately but each as a part of a more or less complicated meshwork. Fig. 63 illustrates the continuity of the water columns through a woody tissue, in which part of the elements have become blocked with air. The air-plugged tracheids or vessel sections become merely "islands" in the meshwork of intact water columns, and the capacity of the tissues as a whole to conduct water, although somewhat impaired by the presence of air in some of the conducting elements, is by no means destroyed.

Magnitude of the Cohesive Force of Water.—If water is to move through the xylem ducts as postulated by this theory, its cohesive force must be of sufficient magnitude to resist the stresses which are imposed upon it. The maximum height to which water ever moves in plants does not exceed 400 feet. This height is equivalent to about 12 atmos. which represents the maximum stress which the water columns could develop as a result of their

own weight. Water, however, encounters a certain amount of resistance in moving through the conducting tissues. The greater the velocity of the current of water, the greater the resistance which it will encounter. Dixon has shown that at the velocity corresponding to usual rates of transpiration this resistance is approximately equal to the pressure required to support water for the height of the plant. He used the wood of the yew (*Taxus baccata*) for his determinations, in which higher values for resistance would be expected than in non-conifers in which most water traverses vessels rather than tracheids. For high rates of conduction Huber (1924) estimates the resistance to be several times as great as this. Accepting Dixon's estimates as an average value, the required cohesive force of water must be doubled, giving a value of 24 atmos. To this must be added the resistance normally encountered by water in crossing the tissues of the root and the mesophyll cells of the leaves which is equivalent to only a few atmospheres. The estimated minimum cohesive force required to lift water to the tops of the tallest tree is therefore about 30 atmos. Even if we assume a resistance three times as great as that found by Dixon, the value of the necessary cohesive force becomes only about 50 atmos.

Values experimentally determined for the cohesive force of water range up to 350 atmos. Dixon obtained values as high as 207 atmos. for sap centrifuged from branches of holly (*Ilex aquifolium*). The cohesive force of water is therefore much in excess of the minimum required for the lifting of water to the tops of even the tallest trees. Although the estimates given in the preceding paragraph represent the minimum cohesive force actually required for the movement of water to the top of tall trees, many botanists believe that when an internal water deficit exists in plants, tensions as great as 100 atmos. or even more may develop in the water columns of at least some species of plants.

One of the most ingenious methods of studying the cohesion of water employs the sporangia of the ferns (Fig. 64, *A*) for this purpose (Ursprung, 1915). Around the edge of a fern sporangium occurs a ring of dead, thick-walled cells, known as the *annulus*. As the sporangium matures water is gradually lost from the cells of the annulus by evaporation. Because of the powerful adhesion between the cell walls and water, the resulting shrinkage in the volume of water in each of these cells results in the thin outer wall of each cell being drawn inward, while the ends of the thicker lateral walls are pulled toward each other. This results in tearing open the weak side of the sporangium, and eventually, in a complete inversion in the position of the annulus, exposing the spores on its outer surface (Fig. 64, *B*). The annulus is now in a condition similar to that of a highly strained spring.

Eventually the cohesive force of the water in the cells of the annulus is exceeded, the water ruptures, and the annulus recoils suddenly into approximately its original position, scattering the spores into the atmosphere by its violent rebound (Fig. 64, *C*). The cohesive force of the water in cells such as these can be measured by allowing the annuli to come to equilibrium with known vapor pressures, and determining the highest vapor pressure at which rupturing of the water occurs. The osmotic pressure corresponding to this vapor pressure (Table 24) is equal to the cohesive force of the water in the annulus cells. Such measurements indicate the cohesive force of the water in these cells to be, at a minimum, between 300 and 350 atmospheres.

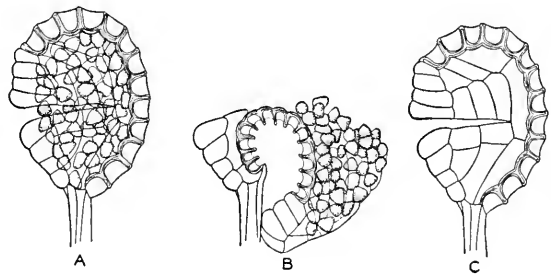

FIG. 64. Behavior of fern sporangium during drying.

The cohesive force of water can also be demonstrated by means of an apparatus such as that shown in Fig. 65. The first experiment of this type was performed by Askenasy (1897). As evaporation proceeds from the porous clay cup of this apparatus water moves up the vertical glass tube, followed by mercury from the reservoir. If the demonstration is successful the mercury will continue to rise above the level to which it will stand in a barometer. The water is now being pulled up the tube, a phenomenon which is possible only because of its cohesive force. The water in the tube is thus thrown into a state of tension which is transmitted to the mercury column below it, due to the relatively enormous adhesive force between water and mercury. The forces exerting this pull originate at the evaporating surface of the cup, and are due to the adhesive and cohesive forces operative in the maintenance of innumerable microscopic menisci in the pores of the clay cup. Thut (1928) succeeded in demonstrating a rise of mercury to a height of 226.6 cm. in such an apparatus. This is approximately three times as high as a column of mercury will be supported by atmospheric pressure acting alone. While the maximum tension developed in the water column in these experiments does not exceed two atmospheres, the demonstration graphically illustrates a

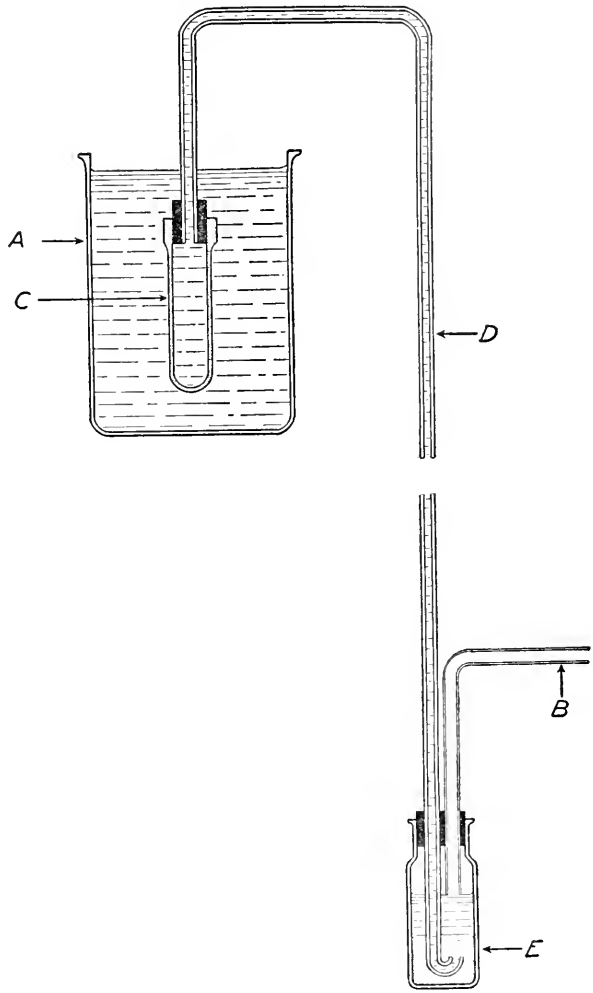

FIG. 65. Apparatus for demonstrating the development of tension in a water column as a result of evaporation. (*A*) beaker of boiled water, (*B*) glass tube, (*C*) porous clay cup filled with water, (*D*) capillary glass tube filled with water, (*E*) mercury layer in bottle. For successful operation it is essential that the water in (*C*) and (*D*) be free of air bubbles. This is accomplished by boiling the water in (*A*) and allowing the hot water to siphon through the apparatus and out at (*B*). To perform the demonstration the beaker (*A*) is removed from around the porous cup (*C*).

physical system which is analogous to that which is believed to operate in the plant.

Experiments similar to that just described have also been performed using small branches or twigs of plants as an evaporating surface instead of a porous clay surface. These are attached at the upper end of a glass tube in place of the clay cup; otherwise the set-up for such an experiment is similar to that just described. While it has not been possible to obtain as great a rise of mercury in these experiments as when a porous clay evaporating surface is used, upward traction of mercury for distances well in excess of atmospheric pressure can be demonstrated if a suitable technique is followed (Thut, 1932).

Development of Tension in the Water Columns.—Convincing evidence that the water in the xylem vessels is often in a state of tension has been obtained by direct observations of vessels under a microscope. The stems of

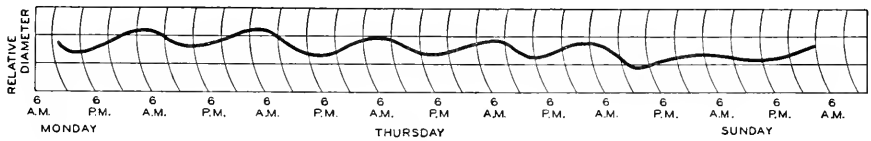

FIG. 66. Daily variations in the diameter of the trunk of a Monterey pine (*Pinus radiata*) as measured with a dendrograph. Data of MacDougal (1936).

some species of herbaceous plants, especially the Cucurbits, are especially suitable for such observations. Such a stem of an intact, rapidly transpiring plant can be fastened in position across the stage of a microscope and, by careful dissection, vessels examined individually. If one of the vessels under observation be jabbed with the point of a fine needle, an immediate jerking apart of the water column at the point of the rupture will be seen to occur, indicating that the water in the intact vessel was actually in a state of tension.

Interesting evidence that the water in the xylem ducts of woody stems is, at times at least, under tension has been obtained by means of an instrument known as the *dendrograph*. This is a self-recording instrument which measures variation in the diameters of tree trunks. It is so constructed that its sensitivity is very great and that its recordings are not influenced by temperature effects upon the instrument. Dendographs are used principally to measure periodic variations in the diameter growth of trees. However, even in trees in which diameter growth has ceased, slight diurnal periodic variations in the diameter of trees have been found to be of regular occurrence.

A record of the periodic variations in the diameter of a tree trunk for a period of several days at a season when little diameter growth was occurring is pictured in Fig. 66. The trunk attained its minimum diameter during the

afternoon hours, at a period when the water columns are undoubtedly under their maximum tension. While under tension the water columns are stretched and decrease in diameter. Due to the enormous adhesive force between water and the walls of the vessels, this results in a slight contraction in the diameter of the vessels. Such diurnal changes in the diameter of a tree trunk are due to alternate contraction of the vessels when the water in them is under tension followed by their dilation when the tension is slackened.

Vessels and tracheids normally contain water at the time of their differentiation and remain filled with water for varying periods of time thereafter. Ultimately most of the water columns in a plant break, but this does not happen to all of them at any one time except under such extreme conditions as prolonged drought. Breaking of water columns occurs principally at times when they are subjected to high tensions.

The Relation of Transpiration to the Movement of Water through the Plant.—In most discussions of the cohesion of water theory of the rise of water in plants, the relation of transpiration to this process is so greatly emphasized that it has come to be almost indelibly associated with this concept. Transpiration is not, however, the fundamental cause of the upward movement of water in plants. Water ascends in the xylem ducts because of and only when a diffusion pressure deficit has been created in the water of the vacuole or the cell walls of the mesophyll cells. Since evaporation of water from the walls of the mesophyll cells is the most frequent cause of such diffusion pressure deficits, the process of transpiration has been generally linked in discussions with the mechanism of the ascent of sap. It is only because of its effect in increasing the diffusion pressure deficit of the water in the mesophyll cells that transpiration sets in motion the entire train of water through the plant. Any other process which results in an increase in the diffusion pressure deficit in the cells at the terminus of any plant axis may also induce the translocation of water. Upward movement of water often continues during the night hours after transpiration has virtually ceased. This lag is due to the residual high diffusion pressure deficit of the leaf cells at the end of the daylight period. Water will continue to enter these cells until they reattain the maximum turgidity of which they are capable under the conditions prevailing. Similarly movement of water will occur into any rapidly growing stem tip because the binding up of water in certain phases of the growth process creates a diffusion pressure deficit in the cells at the top of the growing axis, thus inducing the migration of water towards such centers of growth activity.

At the present time most plant physiologists are agreed that the cohesion theory is a correct representation of the principal mechanism by which water

is transported through plants, be they the tallest of trees or herbs only a few feet in height. It is also generally agreed that "root pressures" play a minor role in this process at least under certain conditions and in some species. There is no doubt that living cells are involved in the phenomena generally described under the term "root pressure" and there is at least a possibility that living cells of the xylem are in some way essential to the maintenance or operation of the cohesion mechanism.

Rates of Movement of Water through Plants.—The rate which water ascends through the xylem ducts may vary from a movement so slow as to be almost imperceptible to a speed of at least 75 cm. per minute (Huber, 1932). By rate is meant the length of the water column which will move past a given point in one minute. In general daily variations in the rate of ascent of water closely parallel daily variations in transpiration rate (Baumgartner, 1934).

Lateral Movement of Water.—A cell to cell lateral movement of water in a radial direction undoubtedly occurs along the vascular rays in the stems of most species of plants. In woody stems there is probably also a lateral movement of water around the stem in a tangential direction. Except in trees in which the "grain" of the wood is twisted, the conducting vessels on one side of the tree generally connect with branches on that side of the tree at their upper extremity, and with roots on the same side of the tree at their lower extremity. If no lateral movement of water occurred in woody stems, it would be expected that removal of the roots from one side of a tree would result in a dearth of water, or perhaps even death of the leaves or branches on that side of the tree.

Experiments have been performed on apple, peach, oak, and other woody species in which the roots on one side of the plant were removed in an attempt to determine whether or not lateral movement of the water occurs (Auchter, 1923). Although the water content and growth of the plants treated in this manner diminished there was no difference in the moisture content of the leaves on the two sides of the tree. Neither did the leaves on the side from which the roots had been removed show any greater tendency to wilt on clear, warm days than those on the other side. These results indicate very strongly that lateral movement of water occurs in woody stems, and that the water conductive system of plants acts as a unit system.

Downward Movement of Water.—There are a number of experiments on record which indicate that downward movement of water can occur in stems. For example, Dixon (1924) found that if the tip of the leaf of a potato plant be cut off under an eosin solution the liquid will descend through the xylem tissue of the leaf, petiole, and stem, and eventually reach the under-

ground organs. In general, however, such an effect is to be expected only when an internal water deficit exists in a plant.

The cohesion theory of the movement of water will account equally well for the conduction of water in either the upward or the downward direction through the plant. If conditions are ever such that the diffusion pressure deficit of the cells of roots or some other basal organ of a plant exceeds that of the apical parts of the same plant a reversal of the direction of movement of water in the xylem will occur. Although downward movement of water can be readily induced in plants under certain experimental conditions, such movements of water are probably of infrequent occurrence in intact plants.

Discussion Questions

1. If the water columns in the vessels are under high tensions at times of rapid transpiration why does not air enter the vessels through the pits in their walls from the intercellular air spaces in the stem?
2. When colored dyes are applied to the cut ends of the topmost branches of a tree they often move downward for considerable distances and out into lateral branches. Is this behavior consistent with the idea that tensions exist in the vessels? Explain.
3. If water is pulled up through the vessels and tracheids of trees by forces produced in the leaves why does not water rise as freely in dead trees as in living trees?
4. Why do the leaves on a dead branch dry up while adjoining branches with living leaves continue to obtain water?
5. What explanations can you suggest to account for the fact that many woody stems will "bleed" in the early spring but show no sign of this phenomenon after the leaves appear.
6. Suggest an experimental set-up by which the direction of the movement of the "transpiration stream" in a plant could be reversed.
7. If the cohesive force of water is so great why is it not held rigidly in place when occupying tubes of small diameter?
8. The "imbibition theory," formerly advocated by some botanists, held that movement of water occurred through the walls of xylem elements as a result of imbibitional forces. How would you undertake to check upon the validity of this theory?
9. Some botanists have postulated that upward movement of water occurs through the stems of trees largely or entirely in the form of water-vapor. Evaluate this hypothesis.
10. Is it logical to assume that since the redwoods are the tallest and largest of trees they must have the most effective water-conducting system?
11. Suppose the primary xylem of the young roots of a twenty year old tree to be injected with a dye. Assuming that no lateral movement of the dye from the xylem to adjacent tissues occurs, trace the exact course of the dye as it rises through the tissues of the root, stem, and leaf.

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CHAPTER XVI

SOILS AND SOIL WATER RELATIONS

Most vascular plants are rooted in the soil from which they obtain both water and mineral salts. The entrance of water from the environment into any of the organs of a plant is called the *absorption, intake*, or *uptake* of water. In most vascular species the quantity of water absorbed through organs other than the roots is of negligible importance. Any thorough consideration of the entrance of water into roots requires a consideration of the properties of soils, particularly as they affect the movement of water from the soil into the root.

Constitution of the Soil.—The soil matrix which is the habitat of most roots is an extremely complex system. In general five different components of this system are distinguished:

1. *The Mineral Matter of the Soil.*—The parent substance of practically all soils is rock, of which there are numerous varieties. By various weathering processes rock strata are reduced to fragments of diverse sizes and these compose the bulk of most soils. The rock particles in soils may vary in size from stones and gravel down to sub-microscopic particles of colloidal clay. The mineral portion of the soil is customarily classified into several fractions depending upon the size of the particles. Such classifications usually disregard the very large particles of the soil (rock fragments, pebbles, etc.). The classification now generally used is as follows:

Fraction	Diameter limits of particles
Coarse sand	2.0 –0.2 mm.
Fine sand	0.2 –0.02
Silt	0.02–0.002
Clay	Less than 0.002

The proportions of the different fractions present are very different in different kinds of soils.

The clay fraction of the mineral portion of soils requires special con-

sideration. Unlike the coarser fractions which are composed of small fragments of unmodified rock minerals such as quartz, feldspar, and mica, the clay portion of soils is made up almost entirely of the products of chemical weathering of the rock minerals and hence differs not only in its physical state, but in its chemical composition from the particles in the coarser fractions. Most of the particles in the clay fraction are of colloidal dimensions and exhibit the characteristic properties of colloidal systems. The particles of the clay complex, like those of many other colloidal systems, retain water by imbibition, *i.e.* within the structure of the particle. On the contrary the sand and silt particles of the soil retain water only on their surfaces. The presence of a considerable proportion of clay in a soil therefore endows it with a high water retaining capacity. Changes in the water content of colloidal clay result in marked changes in its volume. One result of such changes in volume is the commonly observed cracking of a soil rich in clay upon drying. The plasticity and cohesiveness of soils are also due very largely to the colloidal clay present.

Like other colloidal systems colloidal clay is markedly sensitive to the influence of electrolytes. The micelles of colloidal clay usually bear a negative charge when in contact with water. In the presence of calcium ions the individual clay particles are more or less completely flocculated into compound particles. Much of the colloidal clay in most soils exists as enveloping films around the larger soil particles and is also closely associated with organic material in the soil. Flocculation of the clay particles therefore usually results in the formation of compound granules including sand and silt particles and organic matter in addition to the clay. These are called soil crumbs. For agricultural purposes a well developed granular structure of the soil is highly desirable since such a structure favors both a high moisture-retaining capacity and a good aeration of the soil. "Calcium soils," that is, those with a high calcium content, are in general the most suitable and most valuable for agricultural purposes partly because calcium favors the development of a crumb structure. In the presence of an excess of univalent cations (Na^+, etc.), on the other hand, a greater proportion of the clay fraction of the soil disperses into its ultimate particles, and the soil has a single grain structure. In this condition the soil is in the least desirable structural condition for agricultural purposes. Many soils contain hydrogen ions in excess. Such soils often develop a good crumb structure, but the crumbs are less stable than those developed in a calcium soil. The addition of lime improves the physical structure of such soils. The crumb structure of a soil, especially in its surface layers, can also be destroyed by purely mechanical effects, such as trampling, or beating by heavy rains.

2. *The Organic Matter of the Soil.*—Most soils contain organic matter which has been derived principally from the partial decomposition of plant residues. Small quantities may also originate from animal residues and excretions. The proportion of organic matter present may vary from almost none as in some sand deposits to 95 per cent or better in some peat soils. In ordinary agricultural soils the amount present seldom exceeds 15 per cent. In forests organic matter comes from falling leaves, dead branches and trunks of woody plants, roots which die and decay underground, and from dead herbaceous vegetation. In grasslands the underground roots and rhizomes as well as the aerial parts of the plants all contribute their quota to the organic matter of the soil. In well managed agricultural soils attempts are made to maintain the organic matter content by supplying them with organic fertilizers.

The organic matter is the seat of most of the micro-biological processes occurring in the soil. One of the most important of these is the oxidation of the organic matter, a process due largely to the metabolic activities of bacteria and fungi, although a limited amount of purely chemical decomposition probably also occurs. Under conditions which are exceptionally favorable for the activities of micro-organisms the organic matter of the soil is oxidized completely and disappears. For this reason the organic matter of soils in tropical regions, particularly when under cultivation, is very low. Even in more temperate regions cultivation of the soils generally results in a rapid reduction in organic matter content, due principally to the better aeration induced by tillage.

As a result of the decay process there is present in most soils organic matter in various stages of decomposition. A large proportion of the organic material which is added to some soils survives in the form of a dark-colored amorphous substance called *humus*. Humus is composed principally of the degradation products of the cellulose and lignin derived from plant remains. The accumulation of humus in soils is furthered by conditions unfavorable to the oxidative decomposition of organic matter. The decomposition of organic matter in bogs, ponds, swamps, and water-logged soils under conditions which are largely if not entirely anaerobic results in the production of relatively large quantities of humus, frequently of the type which is called peat. Where the organic matter supplied is distinctly acid, as under coniferous forests, or heath plants, humus usually accumulates as a definite layer at the surface. Decomposition of the organic remains under these conditions is largely effected by fungi. In prairie and steppe regions, where a grassland vegetation is predominant, humus usually accumulates in considerable quantities as a result of the decay of both underground and aerial organs of plants.

The amount of humus which accumulates in any soil depends upon the relative rates of the addition of organic residues and of its disappearance as a result of oxidation. Soils with a low humus content may result from a sparse contribution of organic matter, as in desert or semi-desert regions, or from a rapid oxidation of organic material which prevents accumulation of humus even when the supply of organic residues to the soil is large. This latter condition obtains in many tropical and sub-tropical soils.

Humus is essentially colloidal in its properties and possesses an even greater imbibitional capacity than the colloidal clay particles of the soil. The plasticity and cohesiveness of the colloidal organic matter, while considerably less than that of the colloidal clay, is much greater than that of the non-colloidal fractions of the soil. Humus is rather inert chemically, its influence on the soil being largely a physical one. Its presence in soils favors a looser structure and hence better aeration, a higher water holding capacity and a reduction in cohesiveness as compared with heavy clay soils.

Since the clay fraction and the humus are the two essentially colloidal fractions of the soil, and are associated in an intimate physical relationship, many soil scientists refer to these two soil fractions, considered jointly, as the *colloidal complex* of the soil. A large part of this colloidal complex occurs, in many soils, as films enveloping the larger soil particles. The relation of these films to the maintenance of the crumb structure of the soil has already been mentioned. Many other important soil properties are either due to or greatly influenced by the colloidal complex.

3. *Soil Water and the Soil Solution.*—Water is universally a component of soils, although the amount present may vary from the merest trace to a quantity sufficient to saturate the soil, *i.e.* completely fill all of the spaces between the soil particles. Dissolved in the soil water are varying quantities of numerous chemical compounds. These originate principally from the dissolution or chemical weathering of the rock particles, from the decomposition of organic matter, from the activities of micro-organisms, and from reactions between the roots of plants and the soil constituents. It is thus more accurate to speak of the *soil solution* than of the soil water, although in discussions of the water relations of soils it is often a common practice to disregard the presence of solutes in the soil water. The concentration of the soil solution in any given soil varies with the proportion of water present. While in most soils the soil solution is very dilute, in saline and alkaline soils it may be so concentrated that only a few species of plants can survive with their roots in contact with it. The principal cations found in the soil solution are Ca^{++}, Mg^{++}, K^{+}, Na^{+}, Al^{+++}, and Fe^{+++} (or Fe^{++}); the principal anions are HCO_3^{-}, PO_4^{---}, Cl^{-}, NO_3^{-}, SO_4^{--}, and SiO_3^{--}. Other

solutes too numerous to mention may also occur in the soil solution but are usually present only in very low concentrations. Soil water relations receive a more detailed discussion later in this chapter, while the soil solution is discussed more fully in Chap. XXIV.

4. *The Soil Atmosphere.*—The irregularity of the soil particles in size, shape, and arrangement insures the existence of a certain amount of space between them, even in the most tightly packed soils. This is termed the *pore space* of a soil. The pore space of soils varies from approximately 30 per cent of the volume of the soil in sandy soils to about 50 per cent of the volume in clay soils, or even higher in soils which are very rich in organic matter. The pore space of any given soil depends upon the physical and chemical conditions to which the soil is subjected. Conditions favoring a crumb structure of a soil, for example, usually result in an increase in its pore space. The interstitial spaces of a soil may be occupied entirely by air, as in desiccated soils, entirely by water, as in saturated soils, or, as is most usually true, partly by water and partly by air. The relative proportions of water and air present in any given soil vary depending upon the water content of the soil.

The air present in the soil is generally referred to as the *soil atmosphere.* The soil air usually contains a higher concentration of carbon dioxide and a lower concentration of oxygen than the above ground atmosphere. Concentrations of carbon dioxide as high as 5 per cent have been recorded for the soil atmosphere; such values are far in excess of the average value of 0.03 per cent for the air. The accumulation of carbon dioxide in soils is due more to the metabolic activities of micro-organisms than to respiration of soil animals and the underground portions of vascular plants. Except in very dry soils the soil atmosphere is usually saturated or nearly so with water-vapor.

5. *Soil Organisms.*—The soil flora includes bacteria, fungi, and algae. The bacteria are generally the most abundant of all the living organisms present in any soil. Among them are the nitrifying, sulfofying, nitrogen fixing, ammonifying, and cellulose decomposing bacteria. The bacteria accomplishing the oxidative decomposition of cellulose and similar compounds are the most important agents in the production of humus. The numbers of bacteria present vary greatly from soil to soil, and in any one soil vary with seasonal and other fluctuations in soil conditions. Most soils contain between two million and two hundred million individual bacteria per gram of soil. The number of bacteria decreases rapidly with increasing depth, subsoils being sterile or practically so. In general an abundant representation of most species of bacteria is favored in soils by warm temperatures (35 - 40° C.), good aeration, and a good but not superabundant water supply. A high calcium

content of the soil is also favorable to the development of most species of bacteria, apparently because it favors a granular structure of the soil, thus improving aeration. Some species of bacteria, on the other hand, are anaerobic, and thrive when aeration of the soil is deficient. The denitrifying bacteria and certain of the nitrogen-fixing bacteria (*Clostridium* spp.) are examples of anaerobes.

Fungi are, in general, most abundant in soils of acid reaction. In such soils they largely replace bacteria as agents of decomposition of organic matter.

The soil fauna includes protozoa, nematodes, earthworms, insects, insect larvae, and burrowing species of the higher animals. The earthworms are generally credited with having the most important effects on soil structure, at least in many soils. Their activities result principally in a general loosening of the soil, which facilitates both aeration and distribution of water. Many of the other soil animals have similar effects on the structural organization of the soil.

Soil Water Relations under Field Conditions.—In a region of moist climate, if a hole be dug or bored into the ground at almost any place where the soil is deep enough a level at which the soil is completely saturated with water will be encountered at some depth or other. Water will stand in this hole up to this level of complete saturation which is called the *water table*. In river valleys or in close proximity to large bodies of water the water table will usually be reached at a depth of only a few feet under the soil surface. Even in regions of arid climate there are some local situations in which a water table is present. The depth of the water table in any locality usually fluctuates, sometimes very markedly, with seasonal and periodic changes in the relative rates of rainfall, evaporation, transpiration and other factors. Relatively impermeable soil layers sometimes impede downward percolation of water sufficiently to cause the development of temporary water tables which may be far above the level of the true water table. Such temporary water tables have essentially the same effects on soil water relations as true water tables, except that their influence is often only a transient one.

For many years an important role was ascribed to the capillary rise of the water from the water table into the soil above in maintaining the moisture conditions within that soil. Recent investigations have shown, however, that the importance of this source of water in soils has been over-emphasized. Experiments upon the rise of water through columns of soil indicate that water seldom rises through the soil by capillarity from the water table at an appreciable rate for heights of more than a few feet (Keen, 1928). In a typical loam soil the absolute maximum capillary rise of water is about 8 feet (Shaw and Smith, 1927). Such capillary rise as does occur takes place most slowly,

but to the greatest height in clay soils, and most rapidly but to lesser heights in sandy soils, loam soils occupying an intermediate position.

In many soils the water table lies so far below the soil surface that it has little or no effect on the soil moisture conditions in the soil layers which are penetrated by the roots of most plants. Generally speaking even in loam or clay loam soils, a capillary rise of water from a zone of complete saturation is probably ineffective in providing the roots of most species of plants with any considerable part of the water which they absorb unless the water table is within about 15 feet of the surface. Some of the more deeply rooted species may obtain some water from a water table located at depths as great as about 30 feet, but the presence of a water table at greater depths than this is a negligible factor in supplying the roots of any species of plants with water. A capillary rise of water from a water table is usually an important source of water for plants in such locations as river bottomlands or in fields or forests in close proximity to ponds or lakes. In many and perhaps most agricultural soils in moist climate regions plants obtain some water from the water table at least during the earlier part of the growing season. However the widespread practice of drainage of agricultural regions has often resulted in such a marked lowering of the water table as to greatly reduce the possibility that capillary rise of water from below will supply crop plants with any significant part of the water which they absorb.

The other important source of soil water is that part of the precipitation, usually rain, although often melting snow or ice, which percolates downward into the soil. In dry regions this is the only source of water available to plants. Even in more humid regions this is the principal or only source of available soil water in most soils during the dry season of the year. Not all of the rain which falls on the surface of a soil penetrates into it. Some may be lost by surface run-off and a portion usually evaporates before it can sink into the soil. The proportion of the precipitation which is lost in the run-off is in general much less with open, porous soils such as those found in most forests than with those of a lower porosity. A smaller proportion of the water is lost in the run-off when it falls as a gentle rain than when it comes as sudden downpour. The proportion of the precipitation which is lost by evaporation from the surface layers of the soil depends in part on the color, texture, porosity, and other properties of the soil, and in part on the temperature and vapor pressure of the atmosphere, as well as upon the impinging solar radiation.

Let us now consider how percolating rainwater becomes distributed in a soil. We will assume that the soil under consideration is approximately air dry, that it is essentially homogeneous to a depth of several feet, and that

the water table is so far below the soil surface that it has no effect on soil water conditions in the upper layers of the soil. Even an "air dry" soil is not entirely devoid of moisture. Depending upon the type of soil the *hygroscopic moisture*, *i.e.* the water in an air dry soil, ranges from less than 1 per cent of the dry weight of the soil in fine sands to 5 per cent in clay loams. The hygroscopic moisture represents almost entirely water held by imbibition in the cell colloids, or water strongly adsorbed on the surfaces of the soil particles, hence no capillary movement of this fraction of the soil water is possible and it is completely unavailable to plants.

Let us now assume that two inches of rain falls on this soil. After an equilibrium has been attained the soil will be moistened to a certain depth which will vary greatly depending upon the porosity and other properties of the soil. In general the depth of this moist blanket of soil would lie within a range of a few inches in heavy clay soils to perhaps as much as two feet in very sandy soils. In this hypothetical example let us assume that the soil has been moistened to a depth of one foot. The rain which enters the soil through its surface layer increases its water content sufficiently so that capillary distribution of water within the soil begins, capillary movement under such conditions being in the downward direction. The force of gravity also has an influence, but under the conditions as postulated it is so small in comparison with the capillary forces at work that it may be disregarded. As the water becomes distributed through a soil the water films gradually become attenuated until eventually capillary movement of water ceases. When this attenuation of the soil water has reached its limit, water is believed to occupy only the smaller interstices in the soil.

The depth of this moist blanket of surface soil will depend therefore upon the limit to which water can be transferred in the downward direction by capillary movement. Furthermore the water content of this moist layer of soil will be approximately uniform and the boundary between it and the zone of drier soil below will be fairly sharp (Fig. 67, *A*). The attainment of this condition has required the movement of water until it comes to equilibrium with the capillary and gravitational forces influencing its translocation through the soil (Veihmeyer and Hendrickson, 1927).

Following Veihmeyer and Hendrickson (1931) we will use the term *field capacity* as a name for the water content of the moist layer of a soil after an equilibrium has been attained as just described. The field capacity is a critical point in the soil water relations, representing as it does the water content at which further downward capillary movement of water through the soil becomes negligible. Generally speaking the field capacity will range from about 5 per cent in very sandy soils to about 35 per cent in clay loams.

Let us now assume that another two inches of rain falls on this same soil before any appreciable amount is lost from its upper layers by evaporation or transpiration. After a new equilibrium has been attained the upper foot of soil will not have increased in water content, but a layer of the soil approximately two feet in depth will now be moistened up to its field capacity (Fig. 67, *B*). In other words addition of a second increment of water equal in

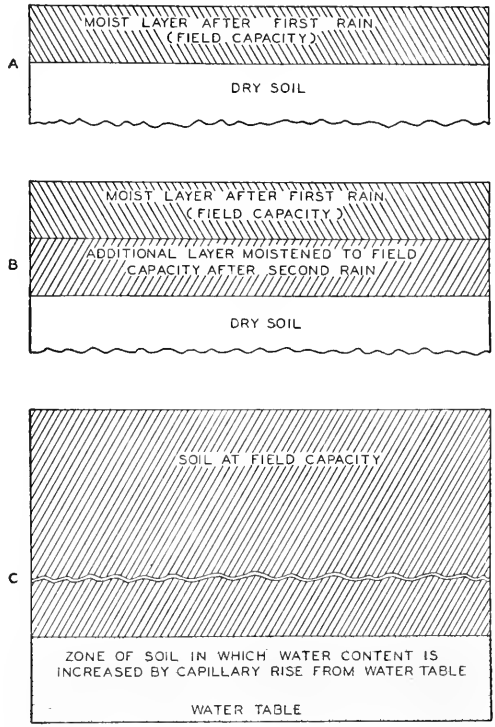

FIG. 67. Diagram illustrating distribution of percolating water in a soil under certain field conditions.

volume to the first does not increase the water content of the already moist layer of the soil above its field capacity, but results, due to further capillary movement, in raising to its field capacity a second layer of soil, lying directly under the zone which had been moistened by the addition of the first increment of rain, and approximately equal to it in thickness. Although the exact mechanics of the distribution of water under these conditions is probably more complicated, the effect produced is as if the second increment of water simply

flows through the moist blanket of soil, after which it is distributed to the dry soil layer underneath by capillarity (Shantz, 1927).

Successive rainfalls would thus continue to deepen the layer of soil which has been moistened up to its field capacity. If the water table is not too deep, and if sufficient rainfall penetrates the soil, not too much of which is utilized by plants growing thereon, eventually the entire soil from its surface down to the water table will be moistened up to its field capacity. A zone several feet in height just above the water table may be enriched somewhat above field capacity by upward capillary movement from the water table (Fig. 67, *C*). If additional water is applied to the soil, after the entire soil mass down to the water table has attained its field capacity, this water percolates down through the soil under the influence of gravity and becomes a part of the ground water. Such conditions obtain in many of the soils of more humid regions at least during the wetter seasons of the year, provided that the water table is not located at too great a depth. This downward percolation of water to the water table is an important factor in influencing its depth below the soil surface, which usually fluctuates considerably from season to season.

In the preceding discussion we have assumed, in the interests of simplicity, a homogeneous soil. However, most soils consist of a vertical succession of several distinct horizons, each with more or less distinctive physical and chemical properties. Even after long continued disturbance of a soil by plowing or other cultural activities at least some semblance of the original soil stratification usually persists. In the horizons below those reached by the plow the original structural organization of the soil is usually maintained practically intact. Although the individual horizons of a soil are often fairly homogeneous within themselves, each such horizon in a given soil may have a distinct field capacity of its own. The water contents of the different horizons, even after an equilibrium in the capillary distribution of water has been attained, may therefore be very different.

A somewhat erratic distribution of rainfall to an underlying soil often results from cracks or channels which are opened into the soil by one agency or another. Many soils crack upon drying, sometimes to depths of several feet. Such cracks often facilitate a mass flow of rainwater to a considerable depth in the soil. Similarly in forest soils the decomposition of roots may leave channels through which water flows down into the deeper layers of the soil. The burrows of animals may also facilitate the entry of water into a soil. The presence of rock strata, or of relatively impervious layers of soil close to the surface, will also modify very markedly the simple picture which has been presented of soil water conditions.

Laboratory Measurements of Soil Water Relations.—The problem of the water relations of soils has been approached both from the standpoint of laboratory and field studies. Some of the broader generalizations resulting from field studies have been just described. Of the numerous laboratory measurements of the water relations of soils which have been devised, only a few of the better known will be discussed.

1. *Water Content.*—The water content of a soil is commonly expressed as the percentage of water present in terms of the dry weight of the soil. It is measured by drying a sample of the soil in an oven to constant weight at (usually) 105° C. and assuming that the loss in weight represents water. There is however nothing critical about the temperature 105° C. The relation between the water content as determined by this method and the temperature at which the soil is dried is a linear one over a wide range of temperatures extending on both sides of this point. This shows that there is no especial significance to be attached to the amount of water which is lost from a soil when dried at this temperature. There is also a small error in such determinations due to some decomposition of organic matter at this temperature.

Determinations of the total water content of the soil contribute very little to an understanding of the absorption of water from the soil by plants. As will be shown later the amount of water available to plants may be very different in two soils of identical water contents.

2. *Water Retaining Capacity.*—One of the most familiar laboratory determinations of soil water is that of the so-called *water retaining capacity* (also called *moisture holding capacity, water holding capacity, water capacity,* etc.). This determination is usually made as follows: A shallow cylindrical pan with a perforated bottom is filled with dry soil, immersed in water to the depth of a few millimeters, and allowed to stand until it becomes saturated. The soil is then allowed to drain free of all the water which will drip out under the influence of the pull of gravity. The water content of the soil after it has been subjected to this treatment, determined by the usual method of oven-drying, is regarded as its water retaining capacity.

It has often been assumed that a determination such as that just described is an indication of the amount of water which would be held by that soil in the field in opposition to the pull of gravity but such an assumption is entirely unjustified. In the first place in making such determinations the vessel is filled with the soil in such a way that the distribution of particles in the soil mass is very different from that which obtains in the field. This error is sometimes minimized by cutting out a core of soil in the field by means of a steel cylinder and using this core for a determination of the water retaining

capacity of the soil. Even this latter procedure does not avoid a more fundamental error which is inherent in the technique of the method itself. When water is allowed to drain from the soil in such determinations the lowest layer of soil particles is in contact—not with the soil as is the situation under field conditions—but with the atmosphere. At the soil-atmosphere interface films of water develop between the soil particles which can only be displaced by a very considerable force. These films interfere with free drainage and cause the accumulation of considerably greater quantities of water in the soil than would occur with free drainage under field conditions. In the field each layer of soil particles is in turn in contact with a lower layer of soil particles and if drainage can occur freely the water content of the soil is ordinarily reduced to the point that capillary movement of the water ceases.

3. *Moisture Equivalent.*—The moisture equivalent is defined as the percentage water content which a soil can retain in opposition to a pull one thousand times that of gravity (Briggs and McLane, 1907). Such a pull is equivalent to a pressure of about one atmosphere. It is determined by placing samples of the soil in especially designed cups with a perforated bottom and whirling them in a centrifuge for (usually) one-half hour (Veihmeyer, *et al.*, 1924). This displaces all of the more loosely held water; that retained by the soil is determined by the usual method of oven-drying, and expressed as a percentage of the dry weight.

The moisture equivalent of a soil is a purely empirical determination, but, except in very sandy or in very heavy clay soils, it closely approximates the field capacity (Veihmeyer and Hendrickson, 1931). Moisture equivalents therefore exhibit about the same range of numerical values as shown by field capacities, ranging from about 5 per cent in very sandy soils to about 35 per cent in clay loams. The principal utility of this determination is that it provides a laboratory measure which closely approximates the field capacity.

4. *Wilting Percentage.*—The foregoing measurements of the water relations of soils are all purely physical determinations. The *wilting percentage* (also called *wilting point* or *wilting coefficient*) is a physiological measure of the water relations of soils. It is defined as the percentage water content of a soil after the plant or plants growing in it have just reached the condition of permanent wilting (Briggs and Shantz, 1912*a*). A permanently wilted plant is usually considered to be one which will not recover its turgidity unless water is supplied to the soil (Chap. XVIII).

In order to determine the wilting percentage of a soil a sample is enclosed in a waterproof vessel. The test plant is generally allowed to develop from seed in the soil sample until it has attained a suitable size. The soil surface is then sealed over so that all loss of water from the system occurs through

the plant. Since no water is added to the soil the plants eventually pass into a state of permanent wilting due to the loss of water by transpiration. As soon as this occurs a sample of the soil is removed and its water content is determined by the oven-drying method.

Prior to extensive determinations of wilting percentages it was supposed that plants differed very markedly in their capacity to reduce the water content of a soil. It was assumed, for example, that species which could endure drought conditions could deplete the moisture content of a soil to a lower percentage before showing permanent wilting than could those species which were soon injured or killed when subjected to drought. Extensive investigations have shown, however, that hydrophytes, mesophytes, and xerophytes all reduce the water content of a given type of soil to about the same value before the condition of permanent wilting is induced (Table 25).

TABLE 25—RELATIVE WILTING PERCENTAGES FOR DIFFERENT SPECIES OF PLANTS (DATA OF BRIGGS AND SHANTZ, 1912*b*)

Species	Wilting percentage	Species	Wilting percentage
Corn	1.03	Coleus	0.99
Wheat	0.994	Potato	1.06
Oats	0.995	Buckwheat	1.05
White sweet clover	1.03	Red beet	1.06
Red clover	1.04	Flax	0.99
Tomato	1.06	Hydrophytes (several species)	1.10
Cotton	1.05	Xerophytes (several species)	1.06

The value of the wilting percentage for each species was determined by calculating the ratio of the individual determination to the mean of all determinations made with that soil. The values given in this table are the average mean ratios for a number of determinations on each species.

While the wilting percentage for a given soil shows no appreciable variation when measured by means of different plants growing in it, the value varies greatly with the type of soil. The percentage of water remaining in a soil when permanent wilting of the plants growing in it occurs ranges from approximately 5 to 10 per cent for sandy loams, from about 10 to 15 per cent for silty loams, and from about 15 to 20 per cent in clay loams.

According to Caldwell (1913) and Shive and Livingston (1914) a greater percentage of water is left in the soil at permanent wilting if the plants are transpiring rapidly than if they are transpiring slowly. Veihmeyer and Hendrickson (1934), however, record that the wilting percentage of a given soil

as determined with sunflower plants shows very little difference over a wide range of climatic conditions. In general this quantity seems to be controlled almost entirely by soil conditions and is only slightly influenced by the species of plant used, or by the climatic conditions to which that plant is exposed.

The significance of the wilting percentage lies in the fact that it is essentially a measure of that fraction of the soil water which is unavailable for plants. However, it should not be assumed that absolutely all movement of water into the roots has ceased when the soil water content has been reduced to its wilting percentage. Wilted plants continue to reduce the water content of the soil but at such a slow rate that restoration of turgidity is impossible since the rate of transpiration even from a wilted plant exceeds the rate of absorption of water from a soil which is at its wilting percentage or lower.

Actually, plants in the field often exhibit permanent wilting at soil water contents somewhat in excess of the wilting percentage as determined by the usual technique. In laboratory determinations of the wilting percentage the plants are ordinarily rooted in relatively small containers in which the soil mass becomes effectively penetrated by roots resulting in a fairly uniform reduction in the water content of the soil. In the field, however, such conditions do not often prevail. The soil is usually less effectively penetrated by the root systems of the plant growing therein, and some portions of the soil may be less completely depleted of water than others before permanent wilting ensues. Although the soil in the immediate vicinity of the absorbing zone of each root is at the wilting percentage more remote portions of the soil may still be at the field capacity. Hence the average water content of such a soil at permanent wilting may be considerably in excess of the wilting percentage as determined by the usual laboratory procedure.

The Diffusion Pressure Deficit of the Water in Soils.—In preceding discussions of the water relations of plant cells and tissues it has been shown that the most significant unit for the expression of the dynamics of water relations of plant cells is the quantity which has been termed in this book the diffusion pressure deficit. Since all problems of the absorption of water by plants involve a consideration of the relation of the water in the soil and the water in the plant, it is desirable that this concept of the diffusion pressure deficit of water be extended to the soil water if the absorption of water is to be interpreted in terms of dynamic units.

One of the first successful attempts to measure the retentive capacity of the soil for water was made by Shull (1916). In an ingenious approach to this problem air-dry seeds of the cocklebur (*Xanthium pennsylvanicum*) were used as the "instrument" for measuring the diffusion pressure deficit of the soil. Seeds of this species were first placed in salt solutions of various

osmotic pressures and the percentage of water which had been imbibed when an equilibrium was attained in each solution was first determined (Chap. IX).

Once the percentages of water held by these seeds when in equilibrium with solutions of different osmotic pressures had been determined, the diffusion pressure deficit of any medium in which they might be immersed could be measured. For example, if some of these seeds were immersed in a medium of unknown osmotic pressure (diffusion pressure deficit) until a dynamic equilibrium was established, after which it was determined that their moisture content was 32.8 per cent, a diffusion pressure deficit of 26.6 atmos. was indicated for the medium (Table 17). This principle was applied to the measurement of the diffusion pressure deficit of soils at different water contents. A sample of soil at a known water content was shaken with some of the seeds until an equilibrium was attained, after which the water content of the seeds was determined.

TABLE 26—DIFFUSION PRESSURE DEFICIT VALUES OF AN "OSWEGO SILT LOAM SOIL" AT DIFFERENT WATER CONTENTS, AS INDICATED BY THE ABSORPTION OF WATER BY COCKLEBUR SEEDS (DATA OF SHULL, 1916)

Soil moisture, per cent of dry weight	Water imbibed by seeds, per cent of air-dry weight	Diffusion pressure deficit of the soil water, atmos.
5.83 ("air-dry")	0.00	965
9.36	6.47	375
11.79	11.94	130
13.16	21.36	72
14.88	28.61	38
17.10	37.70	19
17.93	43.25	11.4
18.07	45.15	7.6
18.87 (approx. wilting percentage)	47.26	3.8

As the data in Table 26 indicate the diffusion pressure deficit of the water in an air-dry soil is of the order of magnitude of 1,000 atmospheres. With an increase in the soil water content its diffusion pressure deficit decreases. In the range of low water contents a small increase in water content corresponds to a very large decrease in the diffusion pressure deficit of the soil. As the water content of the soil is increased further the diffusion pressure deficit continues to decrease but at a progressively slower rate.

Diffusion pressure deficit values for soil water may be calculated from the results of a number of other types of determinations. Among these are vapor pressure measurements (Thomas, 1924), determinations of the freez-

ing point depression of the soil, and measurements of the amount of water retained by a soil in equilibrium with tensions of a known magnitude (Gradmann, 1928). Each of these methods is suitable only for a certain range of soil water contents. The first can be used satisfactorily only with relatively dry soils, the last with relatively wet soils, and the second only over an intermediate range of soil water contents.

From data obtained by one or more of these methods it is possible to construct for any soil a water content-diffusion pressure deficit curve (Fig. 68). Most of the important soil water relations can be interpreted in terms of such curves.

Such a curve is characterized by an abrupt rise in diffusion pressure deficit in the region of low soil water contents. As already shown by the results presented in Table 26, in this zone of soil moisture contents a very small decrease in soil water content corresponds to a very considerable increase in diffusion pressure deficit.

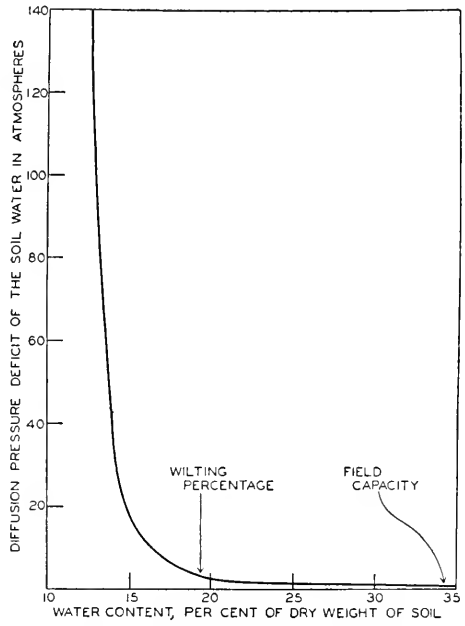

FIG. 68. Relation between soil water content and diffusion pressure deficit of the soil water in a silty clay loam soil. Data of Magistad and Breazeale (1929).

The position of the wilting percentage is just to the right of the sharp inflection which occurs in the curve. The diffusion pressure deficit of the soil water at the wilting percentage lies within a range of 4–12 atmos. Experimentally determined values for this quantity vary slightly depending upon the method used for their measurement. The position of the wilting percentage makes understandable the fact that all species of plants seem to be able to reduce the soil water content of any given soil to approximately the same percentage before they pass into a state of permanent wilting. As shown by this curve reduction of the water content of the soil by only a very small percentage below that of the wilting percentage results in a very rapid rise in the diffusion pressure deficit of the soil. Plants can only obtain water when the diffusion pressure deficit of the soil water is less than that of the peripheral

cells in the absorbing zone of the root. Except in halophytes the diffusion pressure deficit of root cells probably seldom exceeds 25 atmos. and is usually considerably less. Inspection of the curve in Fig. 68 shows that the diffusion pressure deficit of the water in a soil will attain an equilibrium with a plant in which a diffusion pressure deficit of 25 atmos. has developed in the root cells at only a slightly lower soil water content than with a plant in which the diffusion pressure deficit of the root cells is only 5 atmos. Hence wilting percentages as determined with different species of plants are all approximately the same since the actual differences in wilting percentages usually do not exceed the experimental errors inherent in the method of determining this quantity.

To the right of the point corresponding to the wilting percentage the slope of the curve is very gradual. The field capacity corresponds to a diffusion pressure deficit of about one atmosphere, indicating that plants have very little difficulty in absorbing water at soil moisture contents corresponding to this value.

The preceding discussion has been based on the assumption that the soluble salt concentration of the soil is not sufficient to have an appreciable effect on the diffusion pressure deficit of the soil water. In any soil heavily charged with soluble salts, as for example "alkali soils," the diffusion pressure deficit of the water may range up to 100 atmos. or even higher, due to the osmotic effect of the dissolved salts. The curve expressing the soil water content-diffusion pressure deficit relationship in such a soil would not approach very closely to the zero axis, even in soils which are nearly or entirely saturated, but would level off at a value corresponding to the osmotic pressure of the soil solution.

Discussion Questions

1. Why is the water content of a clay soil much greater than that of a sandy soil when both are at the wilting percentage?
2. Why are percentage soil water contents expressed on a dry weight basis, but the moisture percentage of plant tissues usually on a fresh weight basis?
3. List some local habitats in which plants obtain most or all of their water from a water table. Some in which only a part of the water is obtained from a water table. Some in which little or none of the water is obtained from a water table.
4. How could the wilting percentage of a soil be determined if a cactus or other succulent is used as the test plant?
5. What would be some of the effects of tiling a field on the water relations of the soil?
6. A waterproof pot is filled with a known weight of air dry soil with a moisture equivalent of 20 per cent. Water equal in weight to 10 per cent of the dry weight of the entire soil mass is poured on the surface of the soil. How would you expect to find this water distributed in the soil?

7. A greenhouse bench filled with a loam soil is drenched with water. After drainage of water from below has ceased would you expect the water content of the soil to approximate most closely its moisture equivalent or its water retaining capacity?
8. For experimental purposes how would you keep the water content of the soil in a pot at approximately its moisture equivalent?
9. A layer of soil is placed in the funnel of a filter pump, wet thoroughly and then subjected to maximum suction until no further drainage of water occurs from the soil. Will the water content of the soil approximate most closely the field capacity, water holding capacity, or the wilting percentage? Explain.
10. Dust mulches are often recommended as a means of conserving the water in the soil. Evaluate this practice.
11. A corn plant, a young pine tree, and a fern were planted in equal-sized pots containing a silt loam, a sandy loam, and a clay loam, respectively, and the pots sealed against water loss. The soil water was initially at the moisture equivalent in each pot. Assume conditions were favorable to a rather slow rate of transpiration. How will the water contents of the soils compare at the wilting percentage? How will the diffusion pressure deficits of the soil water compare? How will the amounts of water lost from each soil before it reaches the wilting percentage compare? How will the times required for the plants to reach permanent wilting compare?

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CHAPTER XVII

ABSORPTION OF WATER

Roots and Root Systems.—The root system of any species of plant is often as distinctive in form and structure as its aerial portions. Each species has, when growing in its usual or "normal" type of habitat, a characteristic set of roots, just as it has a recognizably distinctive top when growing within its usual range of climatic conditions. Root systems are subject to modifications as a result of the influence of various soil factors, just as the form, height, spread or other features of the tops may be modified in accordance with the climatic condition to which they are subjected. A coniferous tree may produce a stately, cone-shaped crown if growing in a favorable habitat, while another individual of the same species will be only a straggling, scrawny shrub if located near the timber line on a mountain. Similarly, a plant may develop a deep, profusely branched root system in a well-drained soil, while another individual of the same species will produce a shallow root system of entirely different aspect in a soil which is water-logged to within a foot or two of its surface.

Superficially roots resemble stems, inasmuch as both are usually elongate, more or less cylindrical structures. There are, however, a number of important distinctions between the two types of organs. Roots are generally much more irregular in shape than stems. They are not differentiated into distinct nodes and internodes, and hence the branching of roots follows a much less regular pattern than the branching or bearing of lateral organs by stems. With few exceptions the growing tip of every root is capped with a distinctive zone of cells known as the *root cap;* such a tissue is absent from the stem tips. The origin of lateral roots is very different from that of lateral stems; the former develop from deep-seated meristems; the latter from peripheral meristems. The arrangement of the primary tissues in roots is usually different from the arrangement of the primary tissues of stems. Roots bear no appendages which are comparable to leaves or flowers, and lack stomates which are often present in stems.

When a seed germinates the first root which appears is called the *primary root.* This develops from an apical growing region which is already differ-

entiated in the embryo. The primary root, which may be considered as a downward extension of the main axis of the plant, gradually elongates, grows in diameter, and produces lateral branches. Branches and sub-branches of the primary root are called *secondary roots.*

The primary root and its branches considered collectively are called the *primary root system.* In the seed plants primary root systems develop only from embryos. In many species the primary root system remains the only, or at least the conspicuous root system throughout the life of the plant. In perennial plants, especially certain tree species, such primary root systems may attain an enormous size.

All other roots, regardless of the organ of the plant on which they develop, are termed *adventitious roots.* The roots which develop from bulbs, tubers, corms, rhizomes, and cuttings are classed in this category. Adventitious roots may even arise from the leaves of some species such as begonia, bryophyllum, and walking fern. Such roots also develop from the lower nodes of the vertical stems of many species, especially monocots. In some species, maize for example, they may arise from nodes above the soil surface, becoming the so-called prop roots. When they develop from stems adventitious roots most commonly arise at the nodes.

Two very generalized types of root systems which are often distinguished are *tap root systems* and *fibrous root systems.* Practically all adventitious root systems belong in the latter category. In the former the primary root system is predominant, the primary root itself often being conspicuous.

In many species, particularly in the monocot group, the primary root stops growing and may even die while the plant is comparatively young. In such species numerous adventitious roots originate in the region close to the base of the stem resulting in the development of a root system of the fibrous type. Other species of the monocot group, especially many grasses, develop numerous slender adventitious roots from underground rootstocks. Such root systems are also distinctly of the fibrous type.

The root system of a plant is often more extensive than its top. The relative development of the top and roots of a plant is greatly influenced, however, by a number of soil and climatic conditions (See discussion of shoot-root ratio in Chap. XXXIV).

The depth to which roots penetrate into soils is in part a species characteristic, some species being typically more deep-rooted than others. However, prevailing soil conditions usually exert a pronounced effect upon the depth of penetration of roots. Rock strata are frequently so close to the soil surface that the penetration of roots to any great depth is prevented. Similarly the presence of hardpan or otherwise extremely tight layers of soil not far below

the surface checks or at least greatly hinders the invasion of the lower soil layers by roots. If a water table is close to the soil surface downward growth of roots of most species is retarded because of the deficient aeration of saturated soils. Only the roots of hydrophytes, as a rule, can penetrate very far into saturated soils. In dry climates, as for example the western plains area of the United States, the lower limit of root growth is determined by the depth of infiltration into the soil of the scanty rainfall, as generally speaking, roots cannot grow into dry soils. In deep, moist, well-drained soils, on the other hand, the depth of penetration of roots is limited not by soil conditions but by factors inherent within the plant.

Extensive investigations have been conducted by Weaver (1926) and Weaver and Bruner (1927) on the depth of penetration and general distribution in the soil of the roots of many crop plants as well as of some species in their natural habitats. Formerly the concept was prevalent that the roots of crop plants do not penetrate greatly below the depth to which the soil is plowed. Weaver's observations completely refute this idea. He showed that in well-drained soils that the bulk of roots of most crop plants is located in a zone between the surface and a depth of three to five feet. Some individual roots penetrate to greater distances; in most crop plants a few roots were found to reach depths of six or eight feet depending upon the species.

Contrary to popular opinion the roots of trees do not usually penetrate for very great distances into the soil. As a rule most of the root system of the vast majority of trees will be found in the top few feet of the soil. If soil conditions permit a few roots penetrate to greater depths, but growth of tree roots to a depth of more than ten feet beneath the soil surface is uncommon. Trees growing in deep, well-drained soils, especially if sandy or gravelly, may prove exceptions to this statement. Under such conditions the roots of some species, such as cottonwood, may penetrate into the soil for twenty or more feet.

The lateral extent as well as the depth of penetration is an important gross feature of any root system. In general the lateral roots lying close to the soil surface attain the greatest horizontal spread. This varies greatly according to the environmental conditions to which the root system of a plant is subjected. In the more arid climates of the world it is a common observation that the scantier the rainfall the more extensive the lateral development of the root system of many species. Corresponding with this increased lateral development there is usually, under such conditions, a decrease in the depth of penetration.

The density of the vegetation is also a factor of importance in determining the lateral spread of roots. The influence of this factor has been observed

largely in crop plants. Generally a plant which is closely surrounded by other plants will have a more restricted lateral spread to its root system than one growing at some distance from its neighbors. The lateral spread of the roots of a crop plant such as wheat, growing in a dense stand, is always less than that of an isolated plant of the same species. Isolated trees or those growing in open stands often have root systems which extend far beyond the spread of the crown. In artificial tree plantations the density of the stand affects the lateral spread of the roots just as it does in crop plants. The same principle also operates in natural forests but is there complicated by the greater diversity of species and of age groups than is usually present in an artificial planting of trees.

The Absorbing Region of Roots.—Most of the absorption of water and mineral salts occurs in the terminal portions of roots. Measured from the apex, the length of the absorbing region of a root varies greatly with the species and with the conditions under which the root has developed. In general absorption of water and mineral salts can probably take place through any portion of a root which has not become encased in cork cells. As shown in the later discussion, however, there are good reasons for believing that, in at least many species, most absorption of water occurs in the apical portions of roots, and especially in the root hair zone. Because of the extensive branching of roots there are often millions of root tips on the root system of a mature plant. From a physiological point of view the number of root tips borne by a root system is probably the most important index of its effectiveness as an absorbing organ.

The external morphology of a root tip can best be observed in roots which have developed in moist air, peat moss, or sawdust. Upon close examination of a root tip four distinct but intergrading regions can be discerned with no stronger magnification than that afforded by a hand lens, or in many species, even with the naked eye. At the very tip of the root is an extremely short region, white in color, which is known as the *root cap*. Just above the root cap and partly covered by it is the *meristematic region*, the zone of maximum cell division, which is seldom more than a millimeter in length, and which is usually distinguished by a yellowish color. Next in order above the meristematic region is a whiter, smooth region, usually several millimeters in length. This is the *region of cell enlargement* in which most increase in length occurs. Above this region lies the *root hair zone* which bears the slender hair-like outgrowths of the epidermal cells known as the root hairs. The root hair zone varies in length depending upon the species and the conditions to which the root is subjected during its development.

Anatomy of Roots.—If a longitudinal median section be cut through a root tip such as has just been described and examined under a microscope, the anatomy of the several regions of the root can be observed (Fig. 69). The root cap is a more or less thimble-shaped mass of cells covering the end of the meristematic region. It apparently protects the meristematic root tip

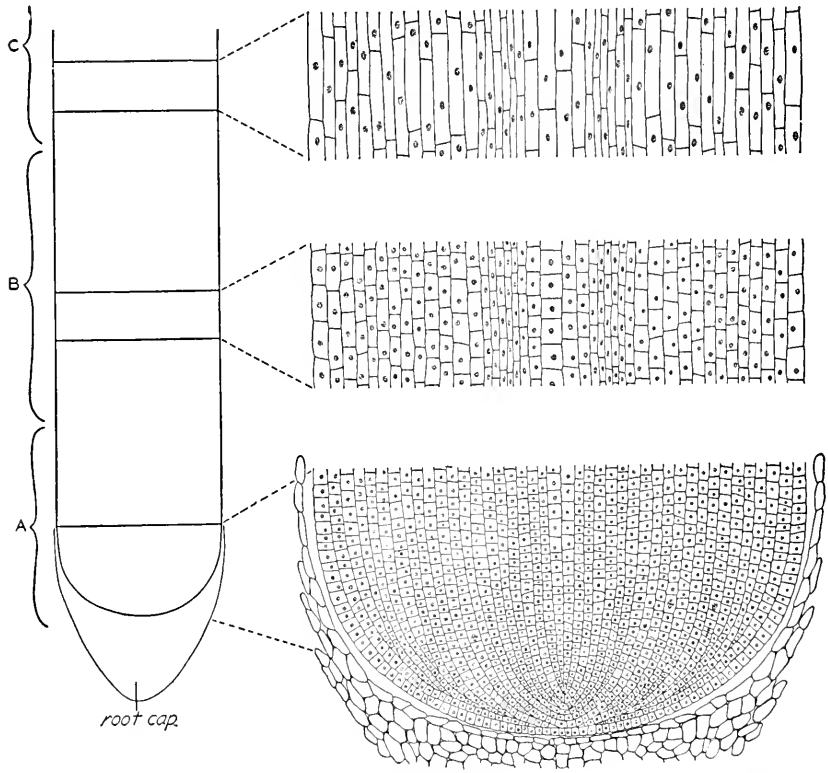

FIG. 69. Longitudinal section (semi-diagrammatic) through a root tip. (*A*) region of cell division, (*B*) region of cell enlargement, (*C*) lower portion of the region of cell differentiation.

from mechanical abrasion as the root grows through the soil. Such abrasion gradually tears off the outer terminal cells of the root cap, but these are replaced by new root cap cells which are formed by cell divisions occurring in the lowermost layers of the cells of the meristematic region.

The meristematic tissue of a root tip is composed of small, thin-walled cells with prominent nuclei. As new cells are formed by cell division they

begin to enlarge, principally in the direction of the long axis of the root. The division of meristematic cells and their subsequent elongation results in projecting the growing region and root cap forward through the soil, and accounts for the growth in length of the roots. The region of cell elongation is seldom more than a centimeter in length, and is usually less. This contrasts markedly with the corresponding region of a stem tip which may be as much as ten centimeters in length, or even longer in some species. Only a small part of the root tip—a few millimeters in length at most—is pushed through the soil. Since, in a growing root tip, the elongation of cells ensues as soon as they are formed by cell division, a cell which is one day in the region of cell division is the next day in the region of cell elongation, subsequent divisions in the meristematic tissue having produced additional layers of cells beyond it.

The anatomy of a representative root as shown in cross section through the root hair zone, is illustrated in Fig. 70. The structure of a young root shows a number of distinctive features. The cortex is relatively thicker than that of stems. This characteristic is especially noticeable in fleshy roots in which the cortex often has a diameter many times that of the stele. The intercellular spaces of the root cortex are also more prominent than those in the stem cortex in which the cells are rather densely packed. An endodermis is almost invariably present in roots; this is generally considered to represent the innermost layer of the cortex. While an endodermis is found in the stems of many species, it is by no means of universal occurrence. Just within the endodermis is present a narrow zone of parenchymatous pericyclic tissue. Usually this is continuous, but in some species, as described below, it may be discontinuous. Lateral roots arise in the pericycle.

In roots the primary xylem and the primary phloem are present in a radial pattern. The primary xylem as seen in cross section appears as a number (usually 2 to 5, although sometimes as many as 20) of radially situated strands. In many roots the center of the stele is composed of xylem; in some, especially monocots and herbaceous dicots, it is composed of pith. Usually the xylem strands terminate in contact with the pericycle, but in some species they abut directly on the endodermis, breaking up the pericycle, as seen in cross section, into a discontinuous series of arcs. The primary phloem of roots occurs as patches of tissue (as seen in cross section) which alternate with the strands of xylem (Fig. 70).

The structure of the individual types of cells occurring in the root tissues is essentially similar to the structure of corresponding types of cells occurring in the stem.

With few exceptions the roots of all perennials and many annuals grow in diameter as they increase in age by means of a cambium layer much as do

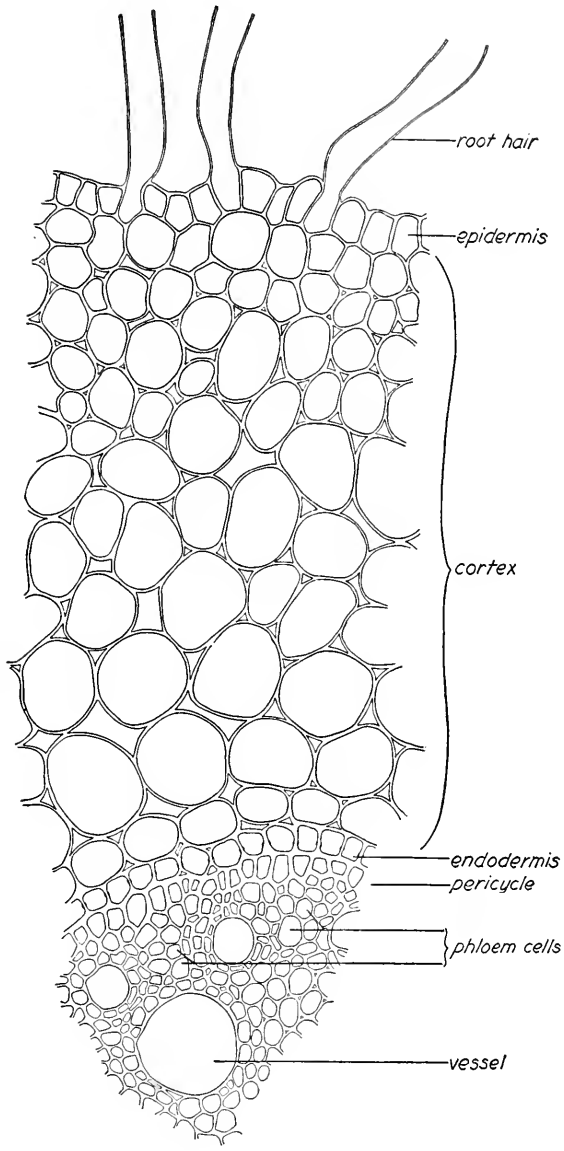

FIG. 70. Cross section of a segment of a young maize root through the root hair zone. Only a portion of each root hair is shown.

most stems. The cambium layer is initiated in young roots in such a way that it lies inside of the strands of phloem tissues, and outside of the xylem strands. In cross section the original cambium layer appears as a wavy band, passing inside of each phloem strand, and outside of each xylem strand. Once differentiated this cambium layer produces secondary xylem on its inside face, and secondary phloem on its outside, just as the cambium of stems does. The initial formation of secondary tissues by a root cambium is usually more rapid in the segments of the cambium internal to the primary phloem strands. Due to this differential growth rate the cambium of a root rapidly attains a circular aspect in cross section. The further differentiation of secondary phloem and secondary xylem proceeds just as it does in stems.

Most perennial roots sooner or later become encased in layers of cork cells. The initial cork cambium often originates in the pericycle. As layers of cork cells are produced by the cork cambium the cortex of the root, including the endodermis, is ruptured and the cells of these tissues die and decay away. Older roots therefore have a characteristic smooth, brownish, corky covering which is pierced only by lenticels. With increasing age, secondary cork cambiums may arise progressively more and more deeply in the phloem tissues. This results in the gradual loss of the pericycle and older phloem tissues. The bark of older roots, therefore, is essentially similar to that of the trunks or larger branches of trees (Chap. XXVIII). Thick layers of bark do not as a rule accumulate on roots as they do on the trunks of some species of trees because of the rapid decay of all dead underground tissues.

In the roots of species in which no secondary thickening occurs, as in many monocots, the epidermal layer of cells may persist intact, usually becoming suberized. In other such species the epidermis may die and decay, but when this occurs an underlying layer of the cortex cells in turn becomes suberized.

Lateral root branches of the primary root system originate in the pericycle, most of them being formed in the region just above the root hair zone. Usually the point of origin of a lateral root is opposite one of the primary xylem strands. The first step in the formation of a lateral root in most species is the development of a group of meristematic cells by the division of several adjacent pericycle cells in the layer just inside of the endodermis (Fig. 71). By successive divisions these cells rapidly form a growing point with its characteristic root cap, region of cell division, etc. As this develops the endodermis and tissues exterior to it are first stretched and later ruptured. The elongating lateral root penetrates through the tissues external to it, partly by mechanical pressure, and perhaps partly by digesting the tissues through

which it passes. Eventually the lateral emerges from the root of which it forms a branch, and becomes an externally visible part of the root system.

Root Hairs.—These structures are confined to the root hair zone, which may be from a few millimeters to many centimeters in length, depending on the species and the conditions under which the root develops. Since the root hair zone lies back of the region of cell enlargement, there is no forward progression through the soil of the individual root hairs along the axis of the root. In any rapidly growing root tip new hairs are continually developing just back of the zone of elongation. New root hairs are thus constantly de-

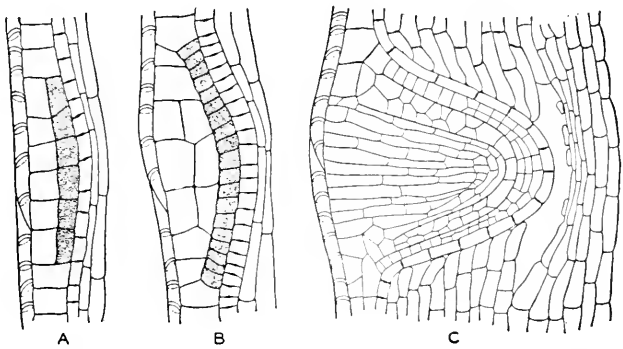

FIG. 71. Three stages in the formation of a lateral root. Meristematic cells (stippled) arise in the pericycle and the cells of the lateral root develop from these. Redrawn from Holman and Robbins (1938) after van Tieghem.

veloping in contact with different portions of the soil, a fact which is of fundamental significance in the absorption of water and mineral salts. Root hairs are often short-lived structures, and frequently die within a few weeks or even less after they develop. In some species (honey locust, redbud, some composites) the walls of root hairs become lignified and they may persist for a year or longer.

Very little information is available regarding the abundance and distribution of root hairs on the root systems of mature plants. Dittmer (1937) found root hairs to be present on all the roots of a four months old rye plant. This plant bore a total of about fourteen billion root hairs. On the other hand some species, especially certain conifers, ordinarily bear no root hairs. The roots of some species, such as maize, produce abundant root hairs when they develop in the soil or in moist air, but few or none when they develop under water as in a solution culture. The roots of many species, on the other

hand, produce root hairs abundantly whether they develop in the soil or in water.

A root hair is essentially a tubular outgrowth of the peripheral wall of an epidermal cell, closed at its distal extremity, projecting more or less at right angles from the long axis of the epidermal cell of which it is an integral part (Fig. 72). Not all of the epidermal cells produce root hairs Root hairs may vary from less than a millimeter up to about a centimeter in length. On many seedlings several hundred root hairs may be borne on a square millimeter of root surface. On some herbaceous species on which such measurements have been made the presence of root hairs on a given area of root increases the exposed surface of that area from 5 to 20 fold.

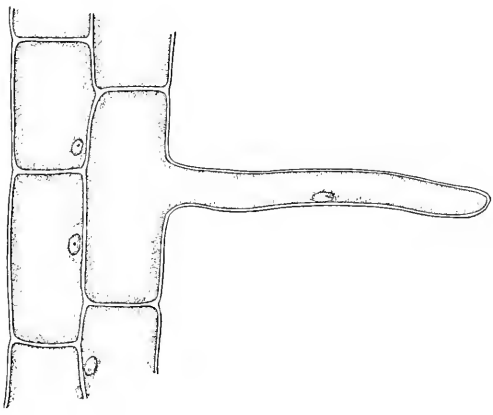

Fig. 72. A young root hair.

The cell wall of a root hair is thin and delicate, and is constructed principally of cellulose and pectic compounds. The outer lamella of the wall seems to be composed entirely of pectic compounds. The tenacity with which root hairs usually adhere to the soil particles with which they are in contact is due to this pectic coating. Due to this intimate contact between the root hairs and soil particles it is difficult to separate the two by washing the root tip or by any other means without severely injuring or destroying most of the root hairs. The inner wall of a root hair is lined with a thin layer of cytoplasm which is continuous with the cytoplasm of the epidermal cell of which the root hair is a part. In water or in moist air the root hairs are usually straight, but in the soil they are more or less contorted, conforming to the shape and distribution of the soil particles among which they penetrate.

The Pathway of Water through the Root.—Water enters the roots through the walls of the root hairs and epidermal cells of the root tip. It then passes through successive rows of the thin-walled cortical cells, and then through the cells of the endodermis (Fig. 70).

The structure of the walls of the endodermal cells is peculiar. Two main types of such cells have been recognized. In one type (Fig. 73, *A*) the inner tangential and radial wall, or sometimes the entire wall, is thickened. These

thickened walls are suberized and sometimes partly lignified. In a second type (Fig. 73, *B* and *C*) a thickened strip is present on the inner surface of the radial and transverse walls, this thickening often being suberized. The width and general configuration of these *Casparian strips*, as they are called, varies with the species. Regardless of type, most thickened endodermal walls are pitted. In some species having the thick-walled type of endodermis, there are present, opposite the outer end of each area of xylem tissue (as seen in cross section) isolated thin-walled endodermal cells called *passage cells*. These

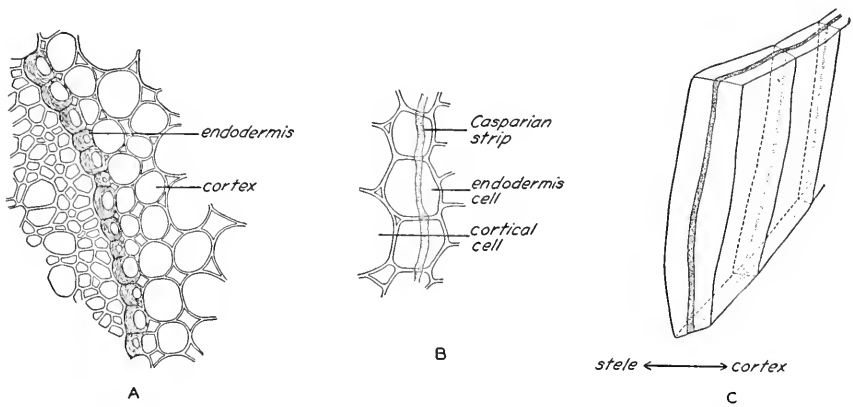

FIG. 73. (*A*) thickened tangential walls of endodermis of a maize root, as seen in cross section. (*B*) Casparian strip in the stem endodermis of *Piper macrophylla*, as seen in cross section. (*C*) perspective diagram showing position of Casparian strip in an endodermal cell.

are supposed to facilitate the movement of water and dissolved salts through the endodermis.

After passing through the endodermis water moves into the xylem ducts, in most species after traversing a few intervening layers of pericyclic cells. The route followed by water through the rest of the plant has already been described in Chap. XV.

The Relation between Roots and Soil Water.—From the standpoint of the absorption of water by plants a clear distinction should be made between soils in which capillary movement of water occurs readily and those in which such movement of water is slow or non-existent. In soils of the former type translocation of water may occur toward the young roots whenever they are absorbing water. There are two principal conditions under which capillary translocation of water can occur in soils at appreciable rates: (1) in any zone of soil which is not more than a few feet above a water table, and

(2) in the upper layers of any soil after a heavy rain or irrigation, but before the water content of the soil has become attenuated to its field capacity. As the water in the films surrounding the soil particles with which the root tips are in contact becomes depleted, more water moves towards those particles by capillarity. The actual rate of such capillary movement of water through the soil may become a factor influencing the rate of absorption. Root systems, however, are not static, but are more or less continually growing through the soil. The rate of root growth of most species decreases, as a general rule, with increasing wetness of the soil above the field capacity, due to the corresponding reduction in soil aeration. Hence in soils in which capillary movement of water occurs—which are necessarily relatively wet—the rate of elongation of roots is generally less than in otherwise similar but somewhat dryer soils. This continued growth of root tips through the soil brings them into contact with other portions of the soil water, so even if capillary movement to certain points ceases, capillary movement of water to the roots may be re-established by the extension of the root tips themselves into zones of the soil which have not yet been depleted of available water.

Many plants, much of the time, grow in soils at water contents between the wilting percentage and the field capacity. In this range of soil water contents capillary movement of water is negligible. Once most of the film water present on the soil particles with which the root tips are in contact has been absorbed it cannot be replaced in any significant quantity by capillary movement from adjacent regions of the soil if the soil water content is below the field capacity. Neither does water move in vapor form through soils towards the absorbing regions of roots at appreciable rates. Under such conditions the absorbing region of every rootlet often becomes surrounded with a narrow cylindrical zone of soil which has been depleted to a water content much below that of the surrounding soil.

Since in soils at a water content below the field capacity movement of water towards the roots is inappreciable, the only method by which the roots come in contact with additional increments of water is by continually growing through the soil (Burr, 1914). Mature root systems of many species of plants bear millions of root tips. Each of these numerous root tips may be pictured as slowly, although often intermittently, progressing through the soil and absorbing most of the water present in the smaller interstices between the soil particles with which they come in contact (Livingston, 1927). That large quantities of water can be absorbed in this way, at least by some species of plants, is shown by the results of Dittmer (1937) on the extent of the root system of a winter rye plant. The total length of all the roots on a four months old plant of this species was found to be 387 miles. This means that,

on the average, each day during the life of this plant the aggregate increase in the length of its root system was more than three miles. Furthermore, new root hairs developed on this plant at an average rate of more than 100,000,000 per day.

This picture of the role of root elongation in the absorption of water (and mineral salts) from the soil is probably an accurate one for many and perhaps most species of plants. The root systems of some species such as orchids, however, are of a stubby, sparingly branched type which would seem to indicate that the quantities of water which they can absorb in the manner just postulated would be relatively small.

The root tip population of any plant is usually so large that often not all of the root tips are subjected to the same soil water conditions. Some may be located in lower soil horizons which contain more water than the upper layers of the soil. At other times, or under other climatic conditions, the reverse situation may prevail. After light rainfalls on a comparatively dry soil, for example, the root tips closer to the surface may be in contact with soil at a higher water content than those at greater depths. Hence some of the root tips are usually absorbing more water as they progress through the soil than others.

As a general rule, however, the water content of any soil must exceed a certain value if roots are to continue to grow through it. Sufficient data are not at hand to define exactly the soil water content at which root growth ceases, but apparently it corresponds approximately with the wilting percentage as would be theoretically expected. The results of some experiments of Walter (1924) substantiate this statement. He found that the rootlets of germinating seeds of water-cress and peas were not able to grow at relative humidities of appreciably less than 98 per cent. Such a value is equivalent to a diffusion pressure deficit of the soil of about 27 atmos. (Table 24) which, in general, would correspond to a soil water content not greatly below that of the wilting percentage. Hendrickson and Veihmeyer (1931) have shown experimentally that roots do not grow into zones of soil which have a water content less than the wilting percentage.

Mechanism of the Absorption of Water.—In Chap. XV it was shown that the development of a diffusion pressure deficit in the mesophyll cells of leaves causes the water in the xylem vessels or tracheids to pass into a state of tension, which is equivalent to an increase in the diffusion pressure deficit of the water in the xylem ducts. As a result of the development of a tension in the water columns a gradient of diffusion pressure deficits is established in the absorbing region of the root, increasing consistently from cell to cell across the root from its peripheral layer to the xylem conduits.

Many authorities believe that the water in the cells in absorbing regions of roots may often pass into a state of tension under conditions such as those just described. If this occurs greater diffusion pressure deficits could develop in the peripheral cell walls of young roots than would otherwise be possible. However, the mechanism as just described will operate even if the water in the root cells never passes into a state of tension. The osmotic pressure of the root epidermal and root hair cells of most species for which measurements are available is about 3–5 atmos. although higher values undoubtedly occur in some species. Hence diffusion pressure deficits of this magnitude can develop in the peripheral cells of roots even if the water in them is never under tension.

Whenever the diffusion pressure of the water in the soil exceeds that of the peripheral walls of the young root cells water will move from the soil into the root. Since the osmotic pressure of the soil solution in most soils is only a fraction of an atmosphere the diffusion pressure deficit of the absorbing cells of a root does not have to be very great before water will enter them from any soil with a water content equal to or greater than the field capacity.

The absorption process which has just been described is often called "passive absorption" because the entry of water into the roots is brought about by conditions which originate in the top of the plant and the root cells seemingly play only a subsidiary role. Although the general picture of this mechanism of absorption which has just been presented is probably correct in its essentials, it is almost certainly oversimplified. The influence of certain environmental factors upon absorption, particularly temperature and oxygen, suggests that the metabolic activities of living cells in the absorption zone of roots also play a role in this process.

The mechanism of absorption just described undoubtedly accounts for the intake of most of the water which enters the roots of plants but it is not the only mechanism of absorption which is known to operate in plants. In many species an internal pressure known as "root pressure" often develops in the xylem (Chap. XV). The occurrence of sap exudation resulting from "root pressure" can be strikingly demonstrated with some species by immersing the root system of a decapitated plant in a potometer (Fig. 39). After a time a dilute sap will begin to ooze from the cut stem, and the absorption of water will be indicated by the movement of the meniscus on the capillary arm of the potometer. If the volume of water exuded is measured it will be found to be not sensibly different from the volume absorbed. In other words water is being absorbed and is moving in an upward direction through the plants as a result of processes which take place in the root cells.

This type of absorption, in which the mechanism involved is localized within the root system, is often called "active absorption." Root pressure

or guttation, and "active absorption" are usually considered to be merely different aspects of the same phenomenon. Since there are many species in which root pressures are not known to occur, this process probably does not occur in some species. "Active absorption" apparently occurs at appreciable rates only when the transpiration rate is relatively low and the soil contains water in abundance.

A number of suggestions have been made regarding the possible mechanism of "active absorption" and root pressure, only one of which will be considered here. The essentials of this theory were first suggested by Atkins (1916). Priestley (1920, 1922) has also advocated a very similar hypothesis. In some species, at least, it can be shown that the osmotic pressure of the sap in the xylem elements of the root is higher than that of the soil solution. Osmotic movement from the soil solution to the xylem might therefore be regarded as occurring through a "multicellular membrane" composed of the intervening cortical cells.

Experimental evidence has been obtained by Kramer (1932) which indicates that the mechanism postulated by this theory to account for "active absorption" and root pressure can be shown to operate in other tissues, and can therefore be regarded as a possible explanation of these phenomena. He showed that when the hollow petioles of the tropical papaw (*Carica papaya*) were used as osmometers, by filling them with a sugar solution and immersing them in water, that water would pass through the living cells of the petiole into a sugar solution with an osmotic pressure of 2 atmos., in spite of the fact that the cells of the petiole themselves had an osmotic pressure of about 9 atmos. The mechanism of such a movement of water may be interpreted in terms of the establishment of a consistently increasing gradient of diffusion pressure deficits from the circumambient water across the cells of the petiole to the enclosed solution.

While a plausible theory can thus be proposed which explains so-called "active absorption' in terms of the simple osmotic movement of water, it is doubtful if such theories are adequate. The rate at which sap is exuded from the xylem in some species seems too great to be explained in terms of the mechanism just described, which necessarily would operate relatively slowly (Crafts, 1936). The findings of Grossenbacher (1938) and others that a more or less regular diurnal fluctuation in root pressure is of common occurrence likewise suggest that a more complex mechanism than the one described is involved. At the best "active absorption" can be only partly explained in terms of simple osmosis, and other mechanisms are almost certainly involved which are not at present understood.

The volume of water passing into plants under the influence of "active ab-

sorption" is, with rare exceptions, relatively insignificant. A potted herbaceous plant, for example, which will exude only a few cubic centimeters of sap per day when decapitated may lose as much as fifty to a hundred cubic centimeters of water per day by transpiration, practically all of which represents "passive absorption."

Factors Influencing the Rate of Absorption of Water.—Any factor which influences the diffusion pressure of the water in the peripheral walls of the young roots or the diffusion pressure of the water in the soil will influence the rate of absorption of water. Furthermore the root system of a plant is more or less continually growing through the soil and when the water content of a soil is less than the field capacity absorption of water at any appreciable rate can occur only if growth of roots through the soil continues. Factors which influence the rate of root growth may therefore also have important effects on the amount of water which can be absorbed.

For reasons which should be clear from the preceding discussion the rate of absorption of water is greatly influenced by the rate of transpiration. Hence any factor which influences the rate of transpiration will indirectly influence the rate of absorption of water. Contrariwise, as already shown in the discussion of transpiration, any factor which influences the rate of absorption will also influence the rate of transpiration. The more important soil factors which influence the rate of absorption of water will now be discussed briefly.

1. *Available Soil Water.*—This term is generally used in a loose way to refer to that fraction of the soil water which can be absorbed by plants. For most practical purposes the available soil water represents that portion in excess of the wilting percentage. Within limits a more rapid rate of absorption of water is possible when the available soil water content is high than when it is low. This is reflected in the more rapid rate of transpiration which may be observed in a plant when the soil is well provided with water as compared with the same or a similar plant growing in a soil deficient in water. As shown in Table 27 the greater the percentage of water in a soil, up to a certain value, the greater the rate of transpiration. The retarding effect of relatively high soil water contents upon the rate of absorption of water is due to the accompanying decrease in soil aeration.

2. *Soil Temperature.*—The influence of soil temperature upon the rate of absorption of water varies greatly with the species. When plants whose roots had developed in solution cultures were transferred from a temperature of about 20° C. to a temperature of 0° C. the rate of absorption was greatly retarded in some species, but scarcely affected in others (Döring, 1935). In

general low temperature had the least affect on absorption of water by the roots of species native to northern latitudes.

TABLE 27—EFFECTS OF VARIATIONS IN THE SOIL WATER CONTENT UPON THE RATE OF TRANSPIRATION OF TOBACCO PLANTS

(Transpiration values are the averages from several plants. The two experiments were not performed concurrently.)

Loam soil with a moisture equivalent of 14 per cent						
Soil water content, per cent of dry weight.....	12	14	16	18	20	22
Transpiration. Gm. per dm.² leaf surface in 82 hours.....	30.4	34.2	36.3	38.5	40.6	39.8
Clay soil with a moisture equivalent of 25 per cent						
Soil water content, per cent of dry weight.....	19	22	25	28	31	
Transpiration. Gm. per dm.² leaf surface in 82 hours.	20.2	26.9	30.4	35.2	33.6	

Sunflower is an example of a species in which the rate of absorption is markedly affected by physiologically low temperatures (Fig. 74). The relation as shown in this figure is that of soil temperature to transpiration rates, but the volume of water transpired can often be taken as an approximate measure of the volume absorbed (See, however, discussion in Chap. XVIII on relative rates of transpiration and absorption). The plants for which these results were obtained were growing in a loam soil with a water content of 28 per cent. As shown in this figure, the transpiration of sunflower plants is practically constant for a soil temperature range of about 55° F. to about 100° F. (about 13°-38° C.), but below this range the rate drops very rapidly. Other experiments by the same investigators showed that the absorption rate approached zero at 0° C. Cotton

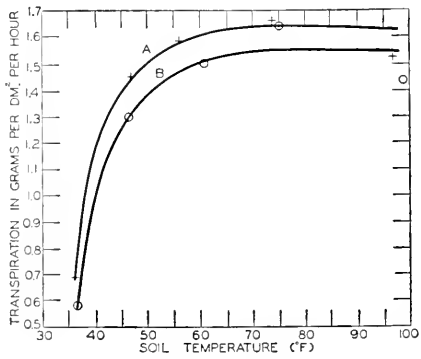

FIG. 74. Relation between rate of transpiration of sunflower plants and soil temperature. Each curve represents the results of a different experiment. Data of Clements and Martin (1934).

is another species in which absorption is soon retarded if the soil temperature is relatively low. Cotton plants wilt when soil temperatures are lowered to between 17 and 20° C. (Arndt, 1937).

The mechanism whereby low soil temperatures cause a retardation in the rate of absorption is undoubtedly a complex one. Among the factors involved are probably: (1) increase in the viscosity of water, (2) decrease in the permeability of the cytoplasmic membranes of the root cells to water, and (3) a diminution in the physiological activity of the root cells. For obvious reasons virtually no water is absorbed by roots from frozen soils.

3. *Aeration of the Soil.*—In general absorption of water by the root systems of most species of plants proceeds more rapidly in well aerated soils than in those which are not. Poorly aerated soils contain a lower concentration of oxygen and a higher concentration of carbon dioxide than the atmosphere. Reduction in the available oxygen supply, at least when severe, reduces the rate of respiration of the roots. If this process is very greatly checked, the rate of root growth and other metabolic processes within the root cells are greatly disturbed. While the roots of most species of plants can survive for short periods in soils practically devoid of oxygen (saturated soils, etc.), as a result of the occurrence of anaerobic respiration (Chap. XXX), maintenance of this process for any considerable length of time by the roots of most species leads to a stunting or even death of the roots. The results of the various physiological disturbances induced by lack of oxygen is a lower rate of absorption of water. Henderson (1934) has shown that there is a close correlation between the rate of absorption of water by maize seedlings and the rate of respiration of their roots.

The retarding effect of poorly aerated soils upon absorption of water may also be due in part to a toxic effect of carbon dioxide on the root cells.

In contrast with other species of plants the roots of hydrophytes normally grow in saturated soils, and absorb water readily from such soils. Some species of hydrophytes have well developed intercellular air passages which lead from the leaves down through the stems into the roots; oxygen undoubtedly moves to the roots through such channels. In other species of hydrophytes, however, no such air conductive system is present. The roots of such species are apparently able to carry on their processes at relatively low dissolved oxygen concentrations.

If a plant with a root system which has developed in the soil is transplanted so that its roots are immersed in a solution culture they will often die within a relatively short time. Flooding the soil in which a plant is rooted often has the same effect. In many species if the root system is allowed to develop from the beginning under water it will grow thriftily in spite of the

inadequate aeration of liquid water or saturated soils. Roots that develop under water can often endure deficient aeration because they are structurally and probably also physiologically different from the roots which develop on individuals of the same species in a well aerated soil. The cortex of "water roots," for example, usually has larger intercellular spaces than the cortex of "soil roots" of the same species.

4. *Concentration of the Soil Solution.*—The concentration of the soil solution of most soils is so slight as to have little or no influence on the diffusion pressure deficit of the soil water, which in most soils, as long as they are fairly moist, does not exceed 1 atmos. In alkaline or saline soils the concentration of the dissolved salts in the soil water is often sufficient to raise the osmotic pressure of the soil solution to a very considerable value—sometimes 100 atmos., or even higher. Intensively cultivated soils, such as those of greenhouses, truck gardens, or irrigated lands sometimes become heavily charged with soluble salts due to the copious application of fertilizers, or to salts brought into the soil by irrigation water.

The diffusion pressure deficit of soils of high solute content, except when relatively dry, *i.e.* below the field capacity, is essentially equal to the osmotic pressure of the soil solution. In general therefore the rate of absorption of water and hence of transpiration is impeded more or less in proportion as the concentration of the soil water solution increases. In solution cultures with an increase in the osmotic pressure of the solution there is a corresponding decrease in the rate of absorption of water by plants. Plants may, within limits, become adjusted to an increased concentration of the soil solution inasmuch as the osmotic pressure of the cells of the plant may increase under such conditions (Table 20). Hence a plant in which the absorption rate is markedly checked when its roots are first brought into contact with a soil solution of higher concentration than the one in which it had been growing, may, after an interval of time, entirely or nearly regain its original rate of absorption of water. Most species of plants can develop normally only when the osmotic pressure of the soil solution does not exceed a few atmospheres. The principal exceptions are species which are native to saline or alkali soils, the so-called halophytes.

Absorption of Water by the Aerial Portions of Plants.—Leaves and other aerial parts of plants are frequently brought into contact with liquid water when raindrops gather on their surfaces, or when dew condenses upon them. Wetzel (1924) found that of a large number of species investigated practically all absorbed some water directly through the epidermis of the leaves. The turgidity of the leaves of a large proportion of the species studied was restored from the wilted condition in 24 hours or less when they were

immersed in water. Absorption of water by leaves under such conditions does not occur through the stomates, but directly through the epidermal cells.

The leaves and other aerial organs of plants may also absorb traces of water-vapor directly from the atmosphere when the latter is saturated or nearly so, but the quantity of water obtained in this way is usually negligible.

Tiny droplets of water are sometimes projected directly through the stomatal pores as a result of splashing during heavy rains, or when leaves are artificially sprayed. Partial or complete injection of the intercellular spaces with liquid water is sometimes brought about in this way. Direct penetration of water in this manner is most likely to happen in species with relatively large stomates such as tobacco.

Discussion Questions

1. How would you demonstrate convincingly that the rate of absorption of water by a plant is markedly influenced by its transpiration rate?
2. Suggest several reasons why relatively low soil temperatures result in low absorption rates even when the temperature of the aerial parts of plants is relatively high.
3. Under what conditions can the transpiration rate be accepted as a fairly accurate indication of the rate of absorption? Under what conditions not?
4. All species of plants reduce the soil to approximately the same water content when the rate of transpiration is slow yet some species are able to deplete the soil of more water than others when transpiration is rapid. Explain.
5. Leaves of tomato plants often recover from wilting more rapidly if the stems are cut and placed in water than when the soil around the roots is well watered. Does this imply that roots are less efficient absorbing mechanisms than the cut end of a stem? Explain.
6. Herbaceous plants with dead root systems absorb water readily from soils with a water content above the moisture equivalent, but quickly wilt if the water content drops below this value. Similar plants with living roots, under identical atmospheric conditions, often wilt in wet soils, but show no signs of wilting in soils below the moisture equivalent. Explain.
7. Why do tobacco plants often exhibit wilting immediately after a heavy rain?
8. A laboratory determination shows the soil in a given field not to be down to wilting percentage yet the plants exhibit permanent wilting. Give possible explanations for this discrepancy.
9. Why does immersion of potted plants of some species in cracked ice often result in wilting while plants of other species can be similarly treated without ever wilting?
10. In a given soil under which of the following conditions would you expect the most extensive root development: (1) at the field capacity? (2) at a water content considerably above the field capacity? (3) close to the wilting percentage? Explain.
11. Why won't seeds germinate in a soil at the wilting percentage when they will absorb water from solutions with a diffusion pressure deficit corresponding to a value less than the wilting percentage (Table 17)?

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CHAPTER XVIII

THE INTERNAL WATER RELATIONS OF PLANTS

In preceding chapters the loss of water from plants, the movement of water through plants, and the absorption of water by the roots of plants have been considered more or less as independent processes, although it should have become increasingly evident as the discussion progressed that the internal hydrostatic system of plants is essentially a unit in its behavior. This point cannot be too strongly stressed as no adequate picture of the water relations of plants can be drawn in terms of these processes considered separately. Many of the more succulent species of plants may, in fact, be regarded as physically little more than a mass of water held in position and shape by an amount of cellular structure which is remarkably small in relation to the volume of water thus retained. Even in woody species of plants, in which the proportion of cellular material to water is greater, the water in the plant is maintained as a continuous unit system. Within this unit system diffusion and mass flow of water are continually in progress. A shift in the diffusion pressure of the water in any part of this unit system will show its influence, sooner or later, but usually within a relatively short time, in other parts of the system. Such effects of changes in the diffusion pressure of the water in one part of this system upon the diffusion pressure of water in other parts of the same system are more pronounced when the rate of absorption of water is slow, or has ceased entirely, than when water is moving into the roots of a plant at a relatively rapid rate.

Wilting.—One of the commonest of observations among agriculturists and gardeners is that the leaves of many species of plants wilt on many hot summer afternoons, only to regain their turgidity during the night even if the plants are not provided with additional water by rainfall or irrigation. In dry, hot regions, or during hot weather in more temperate regions, such a phenomenon may be a regular daily occurrence, even during periods when the soil is well supplied with water. This familiar response of plants is usually called *temporary* or *transient wilting* and is clearly due to a temporary excess of the rate of transpiration over that of absorption. As a result the total volume of water in the plant shrinks, although not equally in all the tissues.

In general diminution of water content is greatest in the leaf cells. The condition commonly called wilting is induced whenever the shrinkage in the volume of water in the leaf cells is sufficient to cause them to lose all or most of their turgor.

Wilting as a visible phenomenon is confined chiefly to species in which the leaf tissues are composed largely of thin-walled, parenchymatous mesophyll cells, and in which the leaves are maintained in their usual firm, expanded condition principally by the turgidity of such cells. External manifestations of wilting can also be observed frequently in young succulent stem tips, floral parts, and even fruits. Root hairs also wilt very commonly, although such wilting usually cannot be observed except under experimental conditions.

In many species of plants the leaves are supported largely by lignified tissues. Examples of species bearing such leaves include many of the evergreens such as pines, holly, mountain laurel, etc. and numerous sclerophyllous species common in the semi-arid regions of many parts of the world. Such leaves wilt just as do parenchymatous ones in the sense that a marked loss of turgor may occur in the leaf cells. The wilting of such leaves is not usually characterized by the drooping, folding, or rolling which are the visible symptoms of wilting leaves composed principally of parenchymatous tissues.

Several stages of wilting are commonly distinguished. Even on days upon which visible wilting does not take place *incipient wilting* is of frequent occurrence. Incipient wilting corresponds to only a partial loss of turgor by the leaf cells. Wilting which becomes visibly apparent in parenchymatous leaves may be of either of the *temporary* or *permanent* types. Temporary wilting, like incipient wilting, is due to a transient excess of the rate of transpiration over the rate of absorption of water. In temporary wilting loss of turgor and resulting volume shrinkage of the leaf cells is so marked as to become visibly discernible in the behavior of the leaves. In the leaves of most species transient wilting corresponds to a complete or nearly complete loss of turgor. Both incipient and transient wilting are to be distinguished from *permanent wilting* which refers to wilting which results, not from a temporary excess of transpiration over absorption, but from an actual deficiency of water in the soil. Plants do not recover from permanent wilting unless the water content of the soil in which they are rooted is increased.

As a general rule the leaves wilt first, because they are the organs from which the great bulk of all water loss occurs, but the decrease in turgor gradually spreads throughout the plant as the internal deficiency of water becomes more severe. Loss of turgor is thus general, although not necessarily equal, throughout all of the tissues of a plant whenever wilting of any considerable duration occurs. Any living cell in a plant may wilt, if this term is used to

designate an approximately complete loss of turgor, which will be the general sense in which it will be employed in this discussion. The longer the condition of wilting persists, the more pronounced such a systemic loss of turgor will be. We may speak, therefore, not only of wilted leaves, or other plant parts, but also of "wilted plants."

The behavior of the stomates during wilting is of especial significance because of the influence of their diffusive capacity upon the rates of photosynthesis and transpiration. As a general rule the stomates close during wilting, although in at least some species their closure is preceded by a transient widening of the stomatal aperture. This passing enlargement in the size of the stomatal pore may be due to a more rapid loss of turgor by the contiguous epidermal cells than by the guard cells, thus permitting a slight further expansion of the latter. Prolonged wilting has been observed to lead to a reopening of the stomates of a number of species. According to Iljin (1932) while a moderate decrease in the water content of a leaf causes condensation of sugar to starch in the guard cells (Chap. XIII), a more pronounced water loss induces the reverse reaction. Hence the diffusion pressure deficit of the guard cells on wilted leaves often attains such a value that movement of water occurs into them even from adjacent cells which are in a flaccid condition. The resulting increase in turgor of the guard cells causes them to open.

Because of its effects on the dynamics of the internal hydrostatic system and upon the stomates, wilting initiates a train of far-reaching effects upon physiological conditions and processes. Even incipient wilting, in which the decrease in the turgor pressure of the cells is relatively small, induces widespread physiological effects within the plant. Some of these have received attention earlier in the discussion of factors influencing the periodicity of transpiration; other such effects, particularly those upon photosynthesis, will receive consideration in later chapters.

Daily Variations in the Water Content of Leaves.—Although a daily variation in the total volume of water present in the body of a plant is a common and almost regular occurrence whenever transpiration is occurring at appreciable rates, because of the experimental difficulties involved no studies have been made of this phenomenon in terms of entire plants. A number of investigations have been made, however, of the diurnal variations in the water content of leaves and other plant organs.

Stanescu (1936) has studied the daily variations in the water content of the leaf blades of Boston Ivy (*Ampelopsis tricuspidata*). The determinations were made on a clear day in early November, but there is no reason for believing that results would be greatly different under "standard day" condi-

tions (Chap. XIII). As shown by his data (Fig. 75) the leaf water content decreased during the morning and early afternoon hours, reaching a minimum at about 5:00 P.M. Thereafter the leaf water content increased, culminating in a maximum which was attained at about 1:00 A.M. During the early morning hours the leaf water content again decreased. Similar observations have been made by Kramer (1937) on leaves of several species. In the sunflower, for example, the minimum leaf water content was attained at about 4:00 P.M., and the maximum at about midnight. The most probable explanation of the occurrence of the maximum leaf water content during the middle hours of the night is that during the early morning hours the leaves

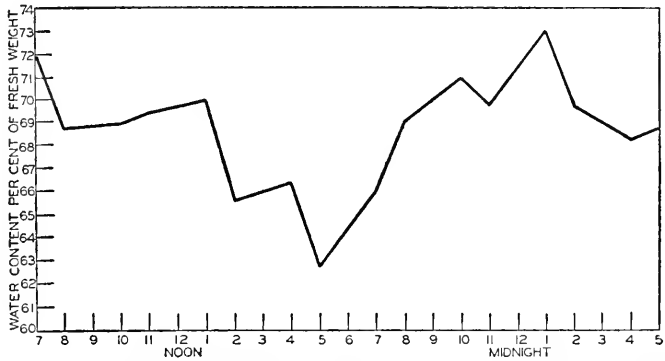

FIG. 75. Daily variation in the water content of leaves of Boston Ivy (*Ampelopsis tricuspidata*). Data of Stanescu (1936).

lose water by translocation to other organs of the plant. The mechanism of such internal redistributions of water in the body of a plant is discussed later in this chapter. Under such conditions the water content of the plant as a whole might be increasing as a result of continued absorption of water, while that of certain organs, such as the leaves, might be diminishing.

The magnitude of the frequently recurrent diurnal reduction in the water content of the leaves and other organs of a plant varies not only with the species, but also with the environmental conditions and their influence on the relative rates of transpiration and absorption. On cool, cloudy or rainy days when the soil is well provided with water often little or no water deficit develops within the plant during the daylight hours. On bright, sunny, but not extremely hot days, while the soil water supply is abundant, an internal water deficit will develop, but it seldom is severe enough to induce more than incipient wilting. On clear, hot days, especially when the soil water supply is not entirely adequate, a more marked shrinkage in the volume of water

within the plant usually occurs which is often of sufficient magnitude to induce temporary wilting. Only if the available water supply in the soil becomes so low that absorption of water virtually ceases will the plant pass into a state of permanent wilting.

The magnitude of the reduction in the water content of the leaves required to induce wilting varies greatly depending upon the species of plant. According to Maximov (1929) leaves of many "sun" species may lose from 20 to 30 per cent of the total water present before wilting ensues, while typical "shade" species wilt upon a reduction in the amount of water present of 3 to 5 per cent. Only in the leaves of the "sun" type can incipient wilting be distinguished as a distinct phase; in "shade" species incipient wilting is extremely transitory. The discussion of wilting and related phenomena in this chapter refers primarily to wilting of the type which is characteristic of plants indigenous to sunny, exposed habitats.

Diminution in the water content of leaves with its concomitant reduction in leaf turgor also influences the total volume of the leaf. The area of leaves may decrease as much as 5 per cent during the midday hours of a bright warm day, the exact magnitude of this shrinkage in area depending upon the species and the prevailing environmental conditions (Thoday, 1909). Not only the area, but even the thickness of the leaves may decrease with a reduction in the turgor of the leaf cells. Bachmann (1922) has shown that a reduction of as much as 5–6 per cent occurs in the thickness of the leaves of many species as they pass from a turgid into a flaccid condition.

Comparative Daily Periodicities of Transpiration and the Absorption of Water.—The observed phenomenon of wilting, as well as experimental results showing that a daily diminution in the water content of leaves and other plant organs is of frequent daily occurrence, are both indirect evidence that the transpiration rate frequently exceeds the rate of absorption of water during the daylight hours. Only a few investigations have been undertaken, however, in which simultaneous measurements have been made of transpiration and absorption rates of the same plant over periods of 24 hours or longer.

For the investigation of this problem Kramer (1937) grew plants in metal containers which were provided with auto-irrigators of porous clay buried in the soil. Each of these irrigators was connected by tubing with a reservoir of water which was set at a lower level than the container. As water is absorbed by the plant from the soil more moves into the soil from the porous clay irrigator which is kept filled by the pressure of the atmosphere on the water in the reservoir. Prior to an experiment the containers were sealed so that all loss of water occurred as transpiration from the plant. By weighing the container plus the reservoir at appropriate intervals and

simultaneously making an observation upon the volume of water which had been absorbed from the reservoir it was possible to make parallel determinations of the rates of transpiration and absorption. Except for the fact that the soil was irrigated the plants were grown under approximately "standard day" conditions.

The results of one of the experiments in which loblolly pine (*Pinus taeda* L.) was used is shown in Fig. 76. As shown in this figure there was a distinct lag in the rate of absorption as compared with the rate of transpiration during the daylight hours—*i.e.* during the period of relatively high transpiration

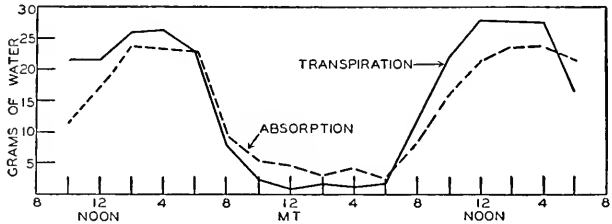

FIG. 76. Comparative daily periodicities of transpiration and absorption in the loblolly pine (*Pinus taeda*). Data of Kramer (1937).

rates. There was also a fairly well marked tendency, shown clearly on the second day of the experiment, for the peak absorption rate to occur somewhat later in the day than the peak transpiration rate. This effect was more pronounced in experiments performed on other species by the same investigator. During the night hours the rate of absorption was continuously higher than the rate of transpiration. In other words the tissues of the plant were being progressively depleted of water during the daylight hours, while their water supply was being replenished at night.

Since the rate of absorption of water in experiments such as that just described is determined by measuring the rate at which water is removed from the external reservoir it is possible that a part of the water deficit observed may have occurred in the soil rather than in the plant. That this source of error is not sufficiently serious to invalidate the conclusions just drawn can be shown by similar experiments in which plants are grown with their roots in potometers adapted to weighing. Even when the roots of plants are immersed in water an internal water deficit develops during periods of high transpiration due to a lag in the absorption rate as compared with the transpiration rate. In all probability in soils in which the water content is at the field capacity or lower, the rate of absorption often shows a more pronounced lag as compared with transpiration than in soils in which capillary

movement of water can occur as is the case when water is supplied by means of auto-irrigators.

In herbaceous plants and probably also in many woody species the lag in the rate of absorption behind the rate of transpiration is largely due to the resistance of the living cells of the root to the passage of water (Kramer, 1938).

Diurnal Variations in the Osmotic Quantities of Plant Cells.—Determinations of daily variations in the water content of leaves and other plant organs have been valuable in elucidating the principle that development of an internal water deficit is a phenomenon of almost daily occurrence in most plants during their growing season. However, such determinations alone are inadequate to give a complete picture of the dynamic aspects of the internal water relations of plants. The same change in water content, for example, which induces a certain shift in the turgor pressure of the leaf cells of one species may have a very different effect upon the turgor pressure in the leaves of another species. In general the influence of fluctuations in the water content upon internal movements of water and upon physiological processes can only be fully interpreted if the status of the water present is expressed in terms of diffusion pressure deficits or other dynamic units.

The daily variation in the osmotic pressure of leaves has already been discussed (Fig. 28). The daily variation in the average diffusion pressure deficit of the leaf cells follows a somewhat similar trend. Under "standard day" conditions (Chap. XIII) the diffusion pressure deficit of the leaf cells is usually low throughout the early morning hours, rises until late afternoon, then decreases during the night hours. During the early morning hours the leaf cells often approach their maximum turgidity. In the late afternoon their turgidity is often low (incipient wilting) and their diffusion pressure deficit approaches the osmotic pressure of the cells (Herrick, 1933). When the diffusion pressure deficit of the leaf cells becomes equal to their osmotic pressure, the turgor pressure of the leaves is zero, and the leaves are in a distinctly wilted condition. As subsequent discussion shows in at least some kinds of plants it is possible for the diffusion pressure deficits of leaf cells to exceed their osmotic pressures.

The increase in the diffusion pressure deficit of leaf cells which is usually observed during the forepart of the daylight period results from the simultaneous operation of the factors of the increasing osmotic pressure and decreasing wall pressure, the latter in turn being due to the gradual diminution in the volume of water in the cells. Similarly the decrease in diffusion pressure deficit which usually begins sometime during the afternoon, and continues during the night hours, is due to the concurrent effect of a decreasing osmotic

pressure, and a gradual replenishment of the water content of the cells, the latter in turn resulting in a progressive increase in the wall pressure of the cells.

Similar, although probably less marked diurnal variations undoubtedly also occur in the osmotic quantities of the cells in the other organs of plants.

Permanent Wilting.—This term refers only to wilting from which a plant will not recover unless the water content of the soil is increased. It is engendered by the development in the soil water of a diffusion pressure deficit so great that the rate of movement of water into the plant is negligible. As with transient wilting, visible symptoms of permanent wilting are apparent only in thin-leaved species of plants, but physiologically equivalent conditions may develop in practically all terrestrial species.

In a soil which is slowly drying out temporary wilting will slowly grade over into permanent wilting. Under such conditions each night recovery of the plant from temporary wilting will occur more slowly and will be less complete, until finally even the slightest nocturnal recovery fails to take place and the plant passes into a continuous state of permanent wilting which grows progressively more drastic the longer that it persists.

Since plants enter the state of permanent wilting by a gradual transition from a condition of temporary wilting, the early stages of this phenomenon are not greatly different from those of transient wilting except that there is no nocturnal recovery of turgor. As the available water in the soil becomes depleted continuity of the soil water with the water in the plant is interrupted, and the water mass in the plant becomes an isolated unit hydrostatic system. When this condition prevails the stress or tension in the hydrostatic system gradually becomes intensified, since, even if the stomates are closed as they usually are in permanently wilted plants, cuticular transpiration continues, thus gradually reducing the total volume of water within the plant. It has been shown experimentally that the water content of the leaves of permanently wilted plants is less than that of plants in a state of transient wilting, and that it gradually decreases during the continuance of permanent wilting. This is also true of the other organs of plants.

Continued gradual reduction in the volume of water in the plant ultimately may throw some or all of the residual mass of water into a state of tension, just as a reduction in the volume of water in a single cell—such as a cell of the fern annulus—has the same effect. A tension generated in the water columns, if of sufficient magnitude, will, in some species at least, be propagated into the leaf cells and other tissues of the plant. Although it is customary to think of the tensions developed in the internal hydrostatic systems of plants largely in terms of the water columns, the existence of water under

tension in plants is not necessarily confined to the conductive system. Continued withdrawal of water from a cell after its turgor pressure has fallen to a zero value can have one of two results; either the water in the cell ruptures or else it is thrown into a state of tension. Tensions of a very considerable magnitude undoubtedly develop in at least some of the cells of many species when subjected to permanent wilting. According to Chu (1936), under conditions of a severe internal water deficiency, the water in the leaf cells of many species of trees, both coniferous and deciduous varieties, passes into a state of tension.

Shrinkage in the volume of water in a cell to the point at which it passes into a state of tension results in the protoplasm and cell walls being subjected to an inward pull because of the strong adhesive force between the water and the cell walls. Under such a condition the wall pressure and the turgor pressure of a cell have a negative value. The greater the tension to which the water in the cell is subjected the greater the pull exerted by the contracted mass of water upon the cell walls. The cell walls of plants are often distorted by the centripetally directed pull which they sustain when the water within them is under tension. It has been observed that the shrinkage in the volume of the cells of a number of species during wilting results in an inward folding or crinkling of the cell walls due to the centripetal pull to which they are subjected (Thoday, 1921; Engmann, 1934). On the other hand the walls of some plant cells are so rigid that they can sustain the development of a considerable tension in the enclosed water without any apparent distortion.

It is generally believed that tensions of a very considerable magnitude can develop in the water columns of permanently wilted plants, probably ranging up to 100 atmos. and perhaps even higher. In some drought resistant species the water columns apparently can be maintained in a state of high tension for weeks or even months without breaking. In many species, however, gradual intensification of the tension in the water columns sooner or later leads to the entrance of air and consequent breaking of the columns.

Internal Redistributions of Water in Plants.—Whenever an internal water deficit develops within a plant the resulting stress in the internal hydrostatic system invariably results in increasing the diffusion pressure deficits in the cells of some organs more than in the cells of other organs. Such a development of unequal diffusion pressure deficits in different parts of a plant frequently leads to the phenomenon often referred to as "internal competition for water" among the various tissues and organs of the plant. This term, although in common use, is not a very satisfactory one, as strictly speaking, tissues within the body of the plant cannot "compete" with each other in any

critical sense of the word. The fundamental concept referred to under this term is essentially that of the migration of water within the plant from regions of its greater to regions of its lesser diffusion pressure. Whenever absorption of water from the soil has ceased, or is occurring at a relatively slow rate as compared with the rate of loss by transpiration, the water in the plant behaves essentially as if it is an isolated hydrostatic system within which water will be continuously moving from regions of its greater to regions of its lesser diffusion pressure. The water in the "competing" organs or tissues is in intercommunication through the continuous water conductive system. Within any plant, therefore, in which the volume of water present has been reduced sufficiently water will migrate most rapidly towards those organs or tissues in which the greatest diffusion pressure deficits have developed.

The actual rate of movement will also be influenced by the resistance of the tissues through which the water must pass. Given a certain diffusion pressure deficit in the cells of a certain organ, water will move more rapidly toward that organ if the resistance offered by the conductive tissues leading to that organ is relatively small than if it is relatively large. The resistance factor affects only the rate and not the direction of the movement of water.

Generally speaking, when a severe internal water deficit develops within a plant, tissues in which the cells possess relatively high osmotic pressures will gain water from tissues in which relatively low osmotic pressures prevail in the cells, since under such conditions the diffusion pressure deficit of a tissue usually approximates its osmotic pressure. Young leaves usually have higher osmotic pressures than older leaves on the same plant, although there are apparently some exceptions to this statement. On most stem axes, therefore, the closer the point of attachment of the leaf to the tip, the greater its omotic pressure in relation to the other leaves on the same axis. The stem tips usually possess relatively high osmotic pressures, equalling or slightly exceeding the values for young leaves on the same plant.

The osmotic pressures of green fruits seem to be invariably less than that of leaves on the same plant (Chandler, 1914). This relation is sometimes although not frequently reversed as the fruit ripens. The osmotic pressures of root cells are in general low as compared with the other tissues of the plant (Hannig, 1912). Some exceptions to this principle have been discovered, however, notably in the garden and sugar beet, in both of which species the osmotic pressure of the "roots" [1] is appreciably in excess of that of the leaves.

The above discussion indicates that under conditions of stress in the internal hydrostatic system the younger leaves and stem tips frequently gain

[1] The "fleshy roots" of beets and a number of other species actually consist principally of enlarged hypocotyls.

water at the expense of older leaves, fruits, and roots. The root hairs are often the first cells to lose water in this way. Maintenance of many plants in a state of permanent wilting for more than a few days usually results in death of the root hairs due to loss of water from them. This is one reason why recovery of many plants from permanent wilting takes place very slowly even after water again becomes available in the soil.

Indications that water often moves from fruits into leaves when an internal water deficit exists in a plant have often been observed. Most such observations have been made upon detached fruit-bearing branches. If two approximately equal-sized cut branches of a lemon tree, for example, one bearing fruits, the other not, be hung up and allowed to dry out the leaves on the fruit-bearing branch will remain fresh and green long after those on the other branch have wilted and withered. Obviously the leaves on the fruit-bearing branch are obtaining water from the fruits. Loss of water from the leaves, although slow, is much more rapid than from the fruits. Due to this

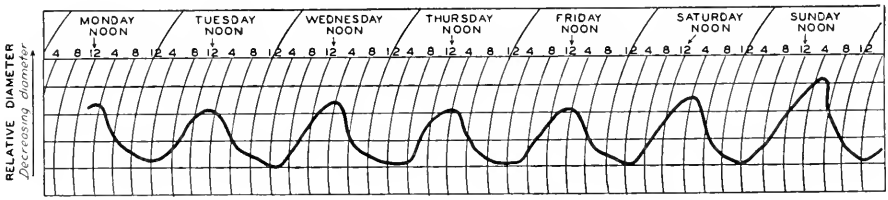

FIG. 77. Daily variations in the diameter of lemon fruits. Data of Bartholomew (1926).

slow transpirational loss the leaf cells soon develop a greater diffusion pressure deficit than the cells of the fruits on the same branch and transference of water from the fruits to the leaves occurs. Concurrently with this loss of water from the fruits a gradual shrinkage in their diameters can be detected. Essentially the same results can be obtained in similar experiments with the fruit-bearing branches of other species.

Similar internal movements of water from fruit to leaves occur in intact plants. One of the most striking investigations of this phenomenon has been made by Bartholomew (1926) who studied the diurnal expansion and contraction of lemon fruits while still attached to the tree. These measurements were made by means of an auxograph, an instrument which automatically records variations in the diameter of fruits and other plant organs.

The auxographic record obtained in one of these experiments is shown in Fig. 77. As shown in this figure the lemon fruit began to contract in volume each day at about 6:00 A.M. and continued to shrink until about 4:00 P.M.

Evidently during this period, which corresponds approximately to the period of relatively high transpiration rates, water was moving out of the fruit into the other organs of the tree. Transpirational water loss from the fruit itself was negligible. Between the hours of about 4:00 P.M. and about 6:00 A.M. the next morning the volume of the lemon gradually increased, showing that a gradual restoration of the water content of the fruit was occurring. Marked daily fluctuations in the diameter of the lemon fruits were apparent even under environmental conditions which resulted in no observable wilting of the leaves. Greater diurnal fluctuations in the diameter of the fruits was found to occur after the trees had gone without irrigation for several weeks than when irrigation water had been recently supplied.

Drought Resistance.—Some species of plants are better able than others to survive and develop in habitats in which a dearth of water is frequent or usual. This capacity of surviving periods of drought with little or no injury is usually termed *drought resistance*. All perennial species of plants native to semi-arid regions are more or less drought resistant. This same statement is true for those species indigenous to local habitats which, for one reason or another, are unduly dry, even in humid climates. Drought resistant species or varieties of plants are important in the agricultural economy of certain regions, such as the "dry-farming" areas of western United States. Certain varieties of crop plants are much more productive in dry regions than other varieties of the same species. Examples are the *durum* and *emmer* varieties of wheats.

The term "drought" is not in itself subject to any rigid definition. In general, however, this term refers to periods during which the soil contains little or no water which is available to plants. In the more humid climates of the world such periods are relatively infrequent and seldom last very long except in certain local habitats. The more arid a climate, in general, the more frequent the occurrence of periods of drought, and the longer their duration. Most species of plants can survive short dry periods without serious injury, but only those possessing a well developed capacity for drought resistance can avoid death or serious injury during prolonged periods of soil water deficiency.

Usually soil drought conditions are accompanied by atmospheric conditions—high temperature, low humidity, and often relatively high wind velocities—which favor high transpiration rates. Such "atmospheric drought" is not only a usual accompaniment of soil drought, but sometimes occurs in the absence of soil water deficiency. Atmospheric drought frequently induces transient wilting of plants during the daylight hours. This alone often results in a serious checking of photosynthesis and growth. In more extreme cases atmospheric drought alone may have devastating effects upon plants.

Hot, dry winds sometimes sweep across grain fields of the western United States, killing or seriously injuring plants, even during periods when the soil water content is still relatively high.

Most species which grow in semi-arid regions, such as the "deserts" of the southwestern United States, or in locally dry habitats, can be conveniently classified into three groups: (1) ephemerals, (2) succulents, and (3) drought-enduring species.

The ephemerals are a prominent feature of the vegetation of all semi-arid regions which are characterized by definite rainy seasons. With the

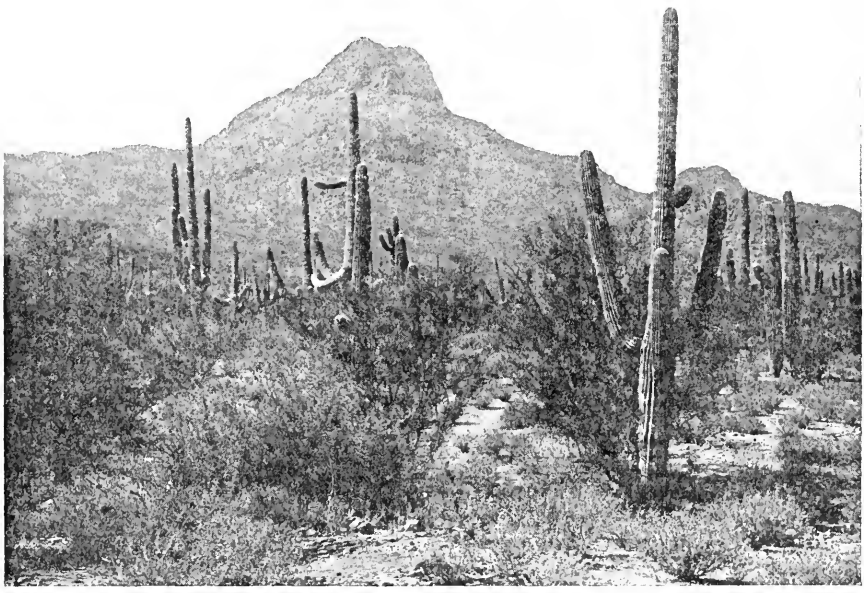

FIG. 78. Semi-desert vegetation near Tucson, Arizona. The prominent succulent is the Sahuaro (*Carnegiea gigantea*). Photograph courtesy of the Desert Laboratory of the Carnegie Institution of Washington.

advent of rains the seeds of such species germinate, and the entire life cycle of the plant is completed within a few weeks. The new crop of seeds survives the intervening dry period until the next rainy season. Such plants have been termed "drought-escaping" (Shantz, 1927). They are no more drought resistant than many mesic annual plants.

Succulents constitute a considerable proportion of the vegetation of most semi-arid regions (Fig. 78), and are frequently found in locally dry habitats

such as sand dunes and beaches in regions of humid climate. The most conspicuous succulents of the American semi-desert regions mostly belong to the cactus family (*Cactaceae*). The other more important families of plants which include a number of succulent species are the *Euphorbiaceae, Liliaceae, Crassulaceae, Aizoaceae,* and *Amaryllidaceae.* The succulents are a distinctive group of plants not only in structure, but in metabolism (Spoehr, 1919) and water economy as well. Species of the succulent habit of growth are able to survive dry periods because of the relatively large reserves of water which accumulate in the inner tissues of the fleshy stems or (in some species) in the fleshy leaves. A relatively thick cuticle and the fact that in many succulents the stomates are generally open only at night are important factors in permitting the conservation of water by such species. Many cacti can live for months on this stored water even if entirely uprooted from the soil. The recent vogue for some of the smaller species of cacti as house plants probably has been favored by the capacity of such species for survival in the arid atmosphere of the average house.

Neither the ephemerals nor the succulents can be regarded as truly drought resistant in the sense that their cells can endure a severe reduction in water content for extended periods of time without injury. This is true only of species which have been classed in the "drought enduring" group. One of the most extreme examples of such a species among higher plants is the creosote bush (*Larrea tridentata* Cav.) which is the dominant plant through large areas of the semi-arid regions of the southwestern United States and northern Mexico. This species carries the same set of leaves through both the wet and dry seasons. During drought periods the water content of the leaves of the creosote bush is sometimes less than 50 per cent of their dry weight (Runyon, 1936). The water contents of the leaves of most woody mesic species, on the other hand, generally range between 100 and 300 per cent of their dry weight.

Some species of plants, including especially many mosses, lichens, and algae, can be reduced to a virtually air dry condition during drought periods, yet remain viable, and resume their life processes very quickly when they are again provided with a supply of moisture. The seeds of many species are drought resistant in this sense that they may be reduced to a nearly air dry condition without losing their viability.

All attempts to explain drought resistance of "drought-enduring" plants upon a purely morphological basis have proved inadequate, although certain structural features of plants undoubtedly aid in their survival in dry habitats. Many xerophytes, for example, have extensive root systems in proportion to their tops. Such root systems may efficiently tap a very considerable volume

of soil, hence the aerial portions of the plant may receive a fairly adequate supply of water even when the rainfall is scanty.

Similarly, many drought resistant species are characterized by relatively small leaves, hence the total area of foliage exposed may be small in proportion to the absorbing capacity of the root system. The leaves of others abscise with the advent of the dry season, and their transpiring surface is thus relatively small during the period of greatest stress upon the hydrostatic system.

The structural peculiarities of xeromorphic leaves such as thick cuticle and hypodermal sclerenchyma are such as to greatly retard cuticular transpiration. Since during drought periods the stomates of xerophytes are closed most or all of the time, the low rate of cuticular transpiration aids in the conservation of the water remaining in the plant.

Formerly it was believed that drought resistant species were characterized by low transpiration rates, and that they are able to withstand drought conditions largely because of their economical expenditure of water. Investigations by Maximov (1929) and others have shown clearly that the transpiration rates of most such species are as great as those of typical mesophytes, whenever the soil water supply is adequate. The frequently observed low transpiration rates of xerophytes are due, not to any inherent peculiarities of structure or physiological behavior, but to the fact that the water content of the soil in which they are rooted is so low that little or no absorption can occur.

Another misconception, long current, was that xerophytes are more efficient in reducing the soil water content than mesophytes. The previous discussion of the effect of the type of plant upon the wilting percentage of a soil indicates that there is no valid evidence for such a belief (Chap. XVI).

As the prior discussion has indicated, during a prolonged period of soil water deficiency the store of water in a plant is gradually depleted, largely as a result of cuticular transpiration. This is true even of xerophytes. One result of this gradual water loss is a progressive increase in the severity of the stress in the internal hydrostatic system. The ensuing gradual dehydration of the tissues sooner or later results in the death of species possessing relatively little drought resistance. Many "drought-enduring" species, on the other hand, can endure this condition, which is physiologically equivalent to permanent wilting, for months at a time without suffering irrecoverable injury.

It seems clear, therefore, that one of the basic factors in the drought resistance of plants is a capacity of the cells to endure desiccation without suffering any irreparable injury. According to Iljin (1930) death of plant cells as a result of drying is not due primarily to the desiccation of the protoplasm, but to the destructive effects upon the protoplasm of various mechanical

disturbances resulting from dehydration of the cell. Purely mechanical effects such as pressure, stretching, tearing, etc. often are destructive to protoplasm. During drying of cells the protoplasm is often subjected to just such effects. The vacuole usually contracts more than the cell wall thus leading to distortion and tearing of the protoplasm. Such drastic disturbances in the protoplasmic system usually result in its death.

As shown by the same investigator cells with a small surface in proportion to their volume and cells in which the size of the vacuole is small relative to the protoplasmic mass are usually less subject to injury during desiccation than cells of structurally opposite types. In cells which fall into one or both of these classes dehydration results in relatively little mechanical deformation of the protoplasm.

In support of his views Iljin (1933) was able to demonstrate that many types of plant cells, including even some from such parenchymatous tissues as the leaf cells of lilac, could be slowly dried out until all water disappeared from their vacuoles and subsequently restored to their turgid condition without killing them. This seems to indicate that desiccation of the protoplasm *per se* is not the cause of the death of plant cells. The turgidity of such cells can be restored without injury only if they are allowed to imbibe water very slowly from concentrated solutions. If immersed directly in water the ensuing rapid absorption results in mechanical deformations of the protoplasm which cause its disorganization. Death of otherwise resistant plant cells apparently may be brought about either by a too rapid drying, or by a too rapid absorption of water while in the desiccated state.

Discussion Questions

1. Why do light showers which do not result in penetration of water to the roots often result in restoring the turgidity of wilted plants? Would this always occur?
2. Why will a wilted potted plant often recover if placed in a saturated atmosphere but sometimes not?
3. Which of the osmotic quantities of the mesophyll cells of a maple tree will show the greatest variation during a twenty-four hour period in midsummer under "standard day" conditions? Why?
4. Leaves of "shade" plants usually wilt upon a reduction in their water content of 5 per cent or less, while most "sun" species do not wilt unless the reduction in leaf water content is 20 per cent or more. Explain.
5. Two similar twigs are cut from a tall tree, one from near the top, the second from one of the lower branches. Both are placed with their cut ends in water. Which will at first transpire more rapidly per unit of leaf area? Would the time of the day at which the twigs were cut make any difference in their relative transpiration rates?
6. At 6 A.M. the water content of leaves on a certain species of plant is found to be 90 per cent; at 4 P.M. on the same day 88 per cent. In terms

of water per gram of dry weight what per cent of the water originally present has been lost?

7. Hollow beech trees usually suffer more severely as a result of prolonged droughts than those with solid trunks. Suggest possible explanations.
8. In lumbering practice it has been found that logs of yellow pine and some other species float more readily if the side branches are not trimmed off until several weeks after the tree is felled than if they are removed immediately. Explain.
9. Why should the tearing of the protoplasm away from a cell wall during drying usually be more harmful to a plant cell than separation of protoplasm from the wall during plasmolysis?
10. Why do the mature leaves of some species of plants vary appreciably in area on days upon which transpiration rates are high while the leaves of other species fail to show such variations?
11. Plot curves which might reasonably be expected to represent the daily variation in the osmotic pressure, turgor pressure, and diffusion pressure deficit of mesophyll cells of a sunflower plant (a) on a "standard day," (b) on a cool, cloudy summer day, (c) on a clear, warm summer day with the soil water content approaching the wilting percentage, (d) on a "standard day" with a heavy thunderstorm in mid-afternoon.
12. In tomato plants the tip of the main stem is usually the last part to die from lack of water, but it is usually the first part to show signs of wilting. Explain.
13. Plot curves which might reasonably be expected to represent the daily variation in transpiration and absorption of water in a corn plant (a) on a "standard day," (b) on an otherwise "standard day" with the soil water content approaching the wilting percentage, (c) on a cool, cloudy day with the soil water content at about the field capacity.
14. Cite some examples of xeric habitats which occur in regions with a generally mesic climate.
15. Would the time of the day at which mature leaves of a green plant attain their maximum water content coincide with the time at which the plant as a whole attains its maximum water content? Explain.

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CHAPTER XIX

THE CHLOROPHYLLS AND THE CAROTINOIDS

Radiant Energy.—An elementary knowledge of the physical properties of light and other forms of radiant energy is essential for a proper understanding of photosynthesis, the synthesis and properties of chlorophyll, and many other plant processes to be discussed in the subsequent chapters of this book. Radiant energy, as judged from some of its properties, appears to be propagated across space as undulatory waves. Ordinary sunlight or "white light" from any artificial source seems homogeneous to the human eye but after it has passed through a prism appears as a spectrum of colors. This dispersion

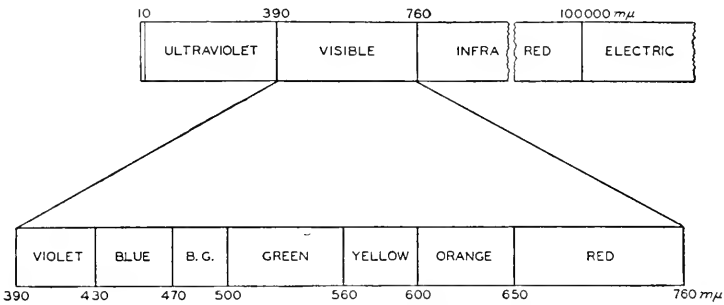

FIG. 79. The spectrum of radiant energy.

of light by a prism was first demonstrated by Newton in 1667, but man had already long been familiar with the similar phenomenon which occurs in rainbows. The order of the more prominent colors in a spectrum of sunlight is red, orange, yellow, green, blue-green, blue, and violet. Each of these colors corresponds to a different range of *wave lengths* of light (Fig. 79). The wave length is the distance between two successive crests of a wave. The wave lengths which induce photochemical reactions in the retina of the human eye that result in the sensation of light, range from about 390 $m\mu$ to about 760$m\mu$.[1]

[1] A millimicron ($m\mu$) is one-thousandth of a micron.

Visible light, however, constitutes only a small part of the spectrum of radiant energy (Fig. 79). Beyond the visible red lies the long zone of infrared or "heat waves" which range up to a wave length of about 100,000 $m\mu$. Electric waves are still longer and range in length up to a kilometer or more. The waves used for radio transmission are in this portion of the spectrum of radiant energy.

Just below the region of visible light in the radiant energy scale lies the ultraviolet zone which ranges down to wave lengths as short as 10 $m\mu$. Even shorter are the X-rays, much used for their therapeutic effects in medicine. Below them on the scale lie the gamma rays which are emitted by radium, also used in medical therapy. Shortest of all are the cosmic rays, which are less than 0.0001 $m\mu$ in wave length.

The wave lengths of the sun's radiation which reach the earth's surface —much of the ultraviolet and infrared are absorbed by the blanket of atmosphere which envelopes the earth—range from about 300 $m\mu$ in the ultraviolet to about 2600 $m\mu$ in the infrared. Only a relatively small portion of this range of wave lengths represents visible light. In their natural habitats plants are also subjected to bombardment by the extremely long electric waves, and the extremely short cosmic waves, but there is no experimental evidence that either of these kinds of radiation has any effect upon plants.

In the preceding discussion we have referred to radiant energy as a wave phenomenon with an air of finality which is not justified by observed facts. Many radiant energy phenomena, such as the behavior of light in optical systems, can only be satisfactorily explained at the present time in terms of the postulate that light travels as waves. Other effects, however, appear completely unintelligible in terms of this hypothesis. The most important of these are photochemical reactions such as the effect of light upon sensitized photographic paper and its rôle in the process of photosynthesis. At the present time such phenomena can only be explained satisfactorily by the assumption that light is particulate in nature. According to this concept a beam of light is pictured as a shower of tiny particles. Each of these particles is called a *photon*. When such photons impinge against a suitable substance their energy may be transferred to the electrons which they strike, thus inducing photochemical reactions.

Each photon carries one *quantum* of energy. The energy value of quanta varies inversely with the wave length. A quantum of ultraviolet radiation with a wave length of 100 $m\mu$, for example, has four times the energy value of a quantum of violet light with a wave length of 400 $m\mu$, and eight times that of a quantum of infrared radiation with a wave length of 800 $m\mu$.

Radiant energy, therefore, apparently possesses a dual nature, and at

present it is impossible to reconcile the corpuscular with the undulatory manifestations of light. All we can say with certainty is that in some of its effects light behaves *as if* it travelled in waves, in others *as if* it is propagated across space as a shower of photons.

The energy value of radiations can be expressed in terms of their equivalent in other forms of energy. On a clear summer's day in the mid-temperate zones the energy value of the impinging solar radiation at noon is usually between 1.2 and 1.5 g.-cal. per square centimeter per minute. This corresponds to an illumination value of about 8,000—10,000 foot candles.

Light and all other radiant energy varies in several different ways, the most important of which are : (1) intensity, (2) quality, and (3) duration. The term "intensity" is often considered to refer to the "brightness" of light, but the two are not the same, as brightness is a measure of intensity only insofar as changes in intensity are registered by the human eye. On the basis of the quantum theory light intensity is considered to depend upon the number of quanta impinging upon a surface of given area per second regardless of the energy content of the quanta. "Quality" refers to the composition of the light in terms of its constituent wave lengths. The quality of the light coming from a tungsten bulb, for example, is relatively richer in infrared radiations and relatively poorer in blue light than sunlight. "Duration" as applied to the light-relations of plants generally refers to the number of hours per day to which a plant is exposed to illumination.

The Chloroplast Pigments.—Green is the distinctive color of the plant kingdom. With only negligible exceptions all leaves are green in color as are also many other plant organs such as herbaceous and young woody stems, young fruits, and the sepals of flowers. The green coloring of plants is often termed simply chlorophyll, although actually, as we shall see shortly, two chemically different chlorophylls can be extracted from plants.

Less evident is the fact that the leaves and many other green organs of plants also contain yellow pigments. These are seldom apparent except in leaves in which the chlorophyll fails to develop or in which it is destroyed as a result of senescence or internal physiological disturbances. Corn plants that have grown from seed in a dark room, for example, do not synthesize chlorophyll but are yellow in color because of the presence of yellow pigments. Similarly the leaves of many woody species become yellow in the autumn. This autumnal change of leaf coloration is due to the disintegration of the chlorophyll which results in an unmasking of the yellow pigments already present. The yellow pigments found in foliage leaves are apparently all either carotene or xanthophylls. Collectively these pigments, together with certain others, closely related chemically, are called the *carotinoids*.

In the higher plants the chlorophylls occur only in the chloroplasts (Chap. VI). The carotene and xanthophylls of leaves are likewise restricted to the chloroplasts. All of these pigments appear to be distributed throughout the proteinaceous ground substance or stroma of the chloroplast. In certain other plant organs such as flower petals these yellow pigments often occur in chromoplasts. The chlorophylls and the associated carotinoids are often called the *chloroplast pigments.*

The Chlorophylls.—Two different chlorophylls have been extracted from plants: Chlorophyll *a*, and chlorophyll *b*. Neither is water-soluble, but both are quite soluble in a number of organic reagents. Chlorophyll *a* is readily soluble in absolute ethyl alcohol, ether, acetone, chloroform, carbon bisulfide and benzol. Chlorophyll *b* is soluble in the same reagents, although generally less so.

Chlorophyll *a* is blue-green in solution, and blue-black in the solid state, while chlorophyll *b* is almost pure green in solution, and greenish-black in the solid state.

Both of the chlorophylls possess the property of *fluorescence.* This term refers to the peculiar property possessed by certain substances when illuminated of re-radiating light of other wave lengths than those falling upon the substance. Usually the radiated light (fluorescent light) is longer in wave length than the incident light. Chlorophyll *a* in ethyl alcoholic solution exhibits a deep blood red fluorescence, best seen by viewing the solution in reflected light. Similar solutions of chlorophyll *b* exhibit a brownish-red fluorescence.

The Chemistry of the Chlorophylls.—Willstätter and his associates have isolated the chlorophylls in pure form from over two hundred different species of plants and found them to be identical in chemical composition. Both chlorophyll *a* and chlorophyll *b* were found in every species studied. They also succeeded in determining the molecular formulas of the two chlorophylls. For chlorophyll *a* this is $C_{55}H_{72}O_5N_4Mg$; for chlorophyll *b* it is $C_{55}H_{70}O_6N_4Mg$. A molecule of chlorophyll *a* has two atoms more of hydrogen and one atom less of oxygen than a molecule of chlorophyll *b*.

Each chlorophyll yields a separate series of degradation products, but one of the products derived from both after mild hydrolysis is an unsaturated primary alcohol which is called *phytol* ($C_{20}H_{39}OH$). Phytol makes up about one-third of the chlorophyll molecule. It has a strong affinity for oxygen and may be responsible for the reducing action of chlorophyll.

When ashed pure chlorophyll leaves a residue composed solely of magnesium oxide. Although iron and other minerals seem essential for the forma-

tion of chlorophyll in living cells, magnesium is the only metallic constituent of the chlorophyll molecule. Chlorophyll contains 2.7 per cent of magnesium.

From the results of the intensive study of the chemistry of the degradation products of chlorophyll the probable structural formulas of both chlorophyll *a* and chlorophyll *b* (Fig. 80) have been determined (Fischer and Breitner, 1936).

As shown in Fig. 80 the nucleus of a molecule of chlorophyll *a* consists of a complex ring structure composed principally of four pyrrol or modified

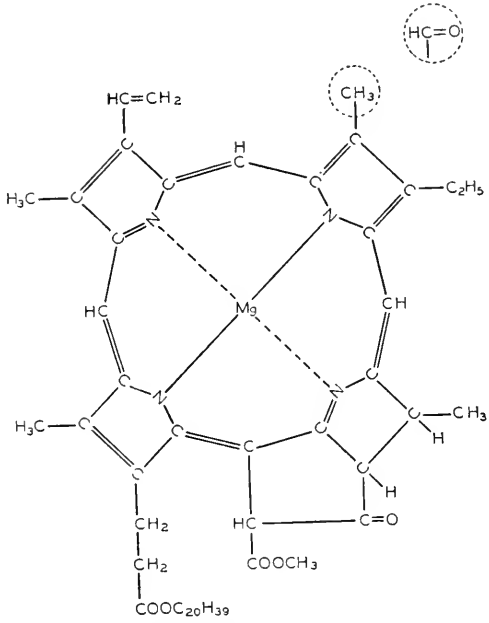

FIG. 80. Structural formula of chlorophyll *a*. The formula of chlorophyll *b* is supposed to be the same except that a CHO group occurs in place of the CH_3 group enclosed in the dotted circle.

pyrrol rings linked together by intermediate atomic groupings. Each such ring bears side chains, the most prominent of which is the phytyl ($C_{20}H_{39}O$—) grouping which upon hydrolysis gives rise to phytol. The position of the Mg atom in the chlorophyll molecule is not known with certainty and its location as shown in this figure is hypothetical.

Absorption Spectra of the Chlorophylls.—When a colored solution such as an ether solution of a chlorophyll is interposed between a source of "white light" and a spectroscope it can readily be shown that certain wave lengths of

light are much more completely absorbed than others. The regions of the spectrum in which complete or nearly complete absorption takes place appear as dark bands. Each of the two chlorophylls shows a definite and characteristic absorption spectrum when in solution. The exact appearance of such absorption spectra will vary, however, depending upon the concentration and the thickness of the layer of solution which is examined. Absorption curves for chlorophyll *a* and chlorophyll *b* in ether solution as determined by photo-

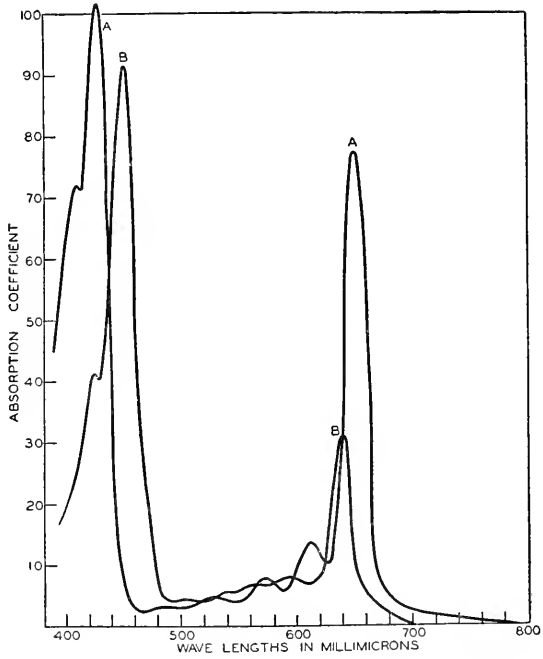

FIG. 81. Absorption spectra of chlorophylls *a* and *b* in ether solution. Data of Zscheile (1934).

electric measurements are shown in Fig. 81. Both chlorophylls exhibit maximum absorption in the blue-violet region and a secondary maximum in the short red.

Physicochemical State of Chlorophyll in the Chloroplasts.—Although the extensive investigations of the physical and chemical properties of chlorophyll extracts have advanced our knowledge of chlorophyll in certain directions, it should be remembered that these are only *extracts* and tell us nothing

regarding the condition of the chlorophyll in the chloroplasts. If we are ever to know how the chlorophyll of living cells operates it will be necessary to discover how it is related to the other constituents of the chloroplast. At least three different views have been expressed regarding the physicochemical state of the chlorophylls as they occur in the chloroplasts: (1) that they are colloidally dispersed in the stroma of the chloroplast, (2) that they are dissolved in a lipoidal solvent which itself may be colloidally dispersed through the stroma, and (3) that they are physically or chemically bound up with proteins or lipids or with both.

The first view has been advanced by Willstätter and Stoll (1913). They adduce two main lines of evidence in its support. In the first place when the absorption spectrum of a solution of chlorophyll is compared with the absorption spectrum of a colloidal sol of chlorophyll dispersed in water it is found that the absorption bands of the colloidal sols are farther toward the red end of the spectrum than those of true solutions of chlorophyll. Since the absorption bands of living green leaves also show a shift towards the red as compared with chlorophyll solutions this is considered to be evidence that the chlorophyll in the living leaf is in the colloidal state. In the second place, perfectly water-free organic solvents will not remove chlorophyll from dried green leaves but in the presence of a small amount of water the extraction readily occurs. Willstätter and Stoll have suggested that the water dissolves small amounts of electrolytes from the leaf which "precipitate" the chlorophyll from its colloidal condition and that the "precipitated" chlorophyll dissolves readily in organic solvents. Chlorophyll can not be removed from the hydrosol condition by the use of the usual solvents unless small amounts of electrolytes are present.

Colloidal sols of chlorophyll dispersed in water do not exhibit fluorescence, differing in this respect from true solutions of chlorophyll. Stern (1920), Lloyd (1924) and others have shown that the chlorophyll in living cells exhibits fluorescence. This is considered to be evidence that the chlorophyll in the chloroplasts is not in a colloidal condition. The results of these investigations have led to the suggestion that the chlorophyll is dissolved in some lipoidal substance which may in turn be colloidally dispersed in the stroma of the plastid.

Noack (1927) considers the chlorophylls to be adsorbed on the protein constituents of the chloroplasts. Since adsorbed chlorophyll fluoresces this is in accord with the observation that the green pigment of living cells exhibits fluorescent properties. It is also possible that the chlorophylls may be present in the chloroplasts in chemical combination with proteins and some evidence in support of this possibility has been obtained (Smith, 1938). In this con-

nection it may be significant that the hemin of the blood, which is chemically similar in many respects to chlorophyll is bound up with the protein globin, forming hemoglobin.

A more extreme viewpoint is adopted by Lubimenko (1926, 1927, 1928) who regards the pigment of the chloroplast to be a complex molecule which is built up of the chlorophylls, carotinoids, and protein. According to this hypothesis the pigments ordinarily extracted from green leaves do not occur in the free state in the chloroplasts but result from the decomposition of a complex type of pigment molecule during the extraction process.

Chlorophyll Synthesis.—Chlorophyll, in common with practically all the other organic substances which occur in plants, is a product of the synthetic activities of the plant. A number of conditions are known to be necessary for or at least to greatly influence the synthesis of chlorophyll in plants. Absence of any one of these factors will inhibit chlorophyll synthesis resulting in the condition often called *chlorosis*. This term is most frequently applied when the failure of chlorophyll to develop is due to a deficiency of one of the essential mineral elements. Different types of chlorosis may develop in the leaves of any species depending upon the factor limiting chlorophyll formation. The factors influencing chlorophyll synthesis will be discussed briefly.

1. *Genetic Factors.*—That genetic factors are necessary for the development of chlorophyll is shown by the behavior of some varieties of maize in which a certain proportion of the seedlings produced cannot synthesize chlorophyll, even if all environmental conditions are favorable for its formation. As soon as the food stored in the seed is exhausted such "albino" seedlings die. This trait is inherited in such strains of maize as a Mendelian recessive and hence is apparent only in plants homozygous for this factor.

2. *Light.*—Light is usually necessary for the development of chlorophyll in the angiosperms. In the algae, mosses, ferns, and conifers, however, chlorophyll synthesis can occur in the dark as well as in the light, although the quantity produced is often less in the absence of light than in its presence. In a few angiosperms such as seedlings of the water lotus (*Nelumbo*), and in the cotyledons of citrus fruits chlorophyll can also develop in the absence of light.

Relatively low intensities of light are generally effective in inducing chlorophyll synthesis in those species in which light is required for this process. All wave lengths of the visible spectrum will, if their energy value is adequate, cause chlorophyll development in etiolated seedlings (chlorophyll-free seedlings which have been grown in the dark) except those longer than 680 $m\mu$ (Sayre, 1928).

It is quite generally considered that chlorophyll is synthesized in the plant

from non-green precursors, although there is much less agreement regarding the exact nature of the chemical changes involved. Lubimenko (1926, 1927, 1928) and others believe that the oxidation of a colorless compound *leucophyll* results in the production of the pigment *chlorophyllogen*. This latter compound, although green, is seldom present in plant tissues in sufficient quantities to impart a color to them. Chlorophyllogen is supposed to be the immediate precursor of chlorophyll. Furthermore it is considered that in the algae, mosses, ferns, and conifers the change of chlorophyllogen to chlorophyll does not require the intermediation of light, but in the angiosperms this transformation seldom occurs except in the presence of light. Under various conditions such as extraction with alcohol or acetone it is further supposed that chlorophyllogen is converted into another distinctive compound called *protochlorophyll*, which is not believed to be directly involved in the sequence of reactions leading to the production of chlorophyll.

Eyster (1928) and Noack and Kiessling (1929, 1930) believe experimental results warrant another interpretation of the chemistry of chlorophyll synthesis. They contend that the pigment protochlorophyll is the immediate precursor of chlorophyll. This compound can be extracted from the inner coats of seeds of certain cucurbits and prepared in a pure form. It exhibits a red fluorescence similar to that of chlorophyll and possesses a prominent absorption band between wave lengths 620 $m\mu$ and 640 $m\mu$. Chlorophyll is believed by these investigators to be an oxidation product of protochlorophyll and the photo-oxidation of protochlorophyll in green cells is believed to be the final step in the formation of chlorophyll in those species in which light is required in this process. Protochlorophyll changes rapidly and quantitatively into chlorophyll on exposure to light.

If a solution of chlorophyll in acetone is exposed to bright light its color soon fades due to a destructive effect of light upon the chlorophyll. This is undoubtedly a photo-oxidation process, since it is accompanied by the absorption of oxygen.

Strong light is also supposed to bring about the disintegration of the chlorophyll in leaves, although at a less rapid rate than in chlorophyll solutions. In leaves exposed to intense light, therefore, synthesis and decomposition of chlorophyll are probably going on simultaneously. In accord with this concept are the results of Shirley (1929) who found in a number of species of plants that the chlorophyll content per unit leaf weight or per unit leaf area increased with decreasing light intensity until a relatively low intensity was reached. Further decrease in light intensity below this value caused a decrease in chlorophyll content.

3. *Oxygen.*—In the absence of oxygen etiolated seedlings fail to develop

chlorophyll even when illuminated under conditions otherwise favorable for chlorophyll formation. This is in accord with the concept that the chemical transformations leading to the formation of chlorophyll from its precursors involve oxidation processes.

4. *Carbohydrates.*—Etiolated leaves which have been depleted of soluble carbohydrates fail to turn green even when all of the other conditions to which they are exposed favor chlorophyll synthesis. When such leaves are floated on a sugar solution, sugar is absorbed and chlorophyll formation occurs rapidly. A supply of carbohydrate foods is therefore essential for the formation of chlorophyll.

5. *Nitrogen.*—Since nitrogen is a part of the chlorophyll molecule it is not surprising to find that a deficiency of this element in the plant retards chlorophyll formation. Failure of chlorophyll to develop is one of the commonly recognized symptoms of nitrogen deficiency in plants.

6. *Magnesium.*—Like nitrogen this element is also a part of the chlorophyll molecule. Deficiency of magnesium in plants results in the development of a characteristic mottled chlorosis of the older leaves (Chap. XXV).

7. *Iron.*—In the absence of iron in an available form green plants are unable to synthesize chlorophyll and the leaves soon become blanched or yellow in color (Chap. XXV). While not a constituent of the chlorophyll molecule, iron is essential for its synthesis. The essential role of this element is believed by some investigators to be an accelerating effect upon the photo-oxidation of protochlorophyll to chlorophyll.

8. *Manganese.*—This element, like iron, seems in some way to be essential in the production of chlorophyll. In the absence of manganese a characteristic mottled chlorosis develops in the younger leaves (Chap. XXV).

9. *Suitable Temperature.*—According to Lubimenko and Hubbenet (1932) chlorophyll synthesis in etiolated wheat plants takes place only within a temperature range of about 3 to 48° C. The maximum rate of chlorophyll formation lies between 26 and 30° C. Temperature affects the rate of chlorophyll synthesis because of its controlling influence upon the rate of transformation of some of the precursors of chlorophyll. The final transformation to chlorophyll from its immediate precursor apparently is a purely photochemical reaction which is uninfluenced by temperature.

10. *Water.*—Desiccation of leaf tissues not only inhibits synthesis of chlorophyll, but seems to accelerate disintegration of the chlorophyll already present. A familiar example of this effect is the browning of grass during droughts.

The mechanism of chlorophyll synthesis is very sensitive to any type of physiological disturbance within the plant. Many other conditions besides

those already discussed, such as lack of certain other mineral elements (potassium, phosphorus, calcium, etc.), deficient aeration of the roots, attacks of insects, bacterial, fungous, or virus diseases, etc., may induce, directly or indirectly, partial or complete chlorosis of the leaves. The failure of chlorophyll synthesis to take place normally is often one of the first observable symptoms of almost any derangement in the metabolic conditions within a plant.

The Carotinoids.—Carotene is found in all green tissues, in the roots of carrots, and in many flowers, fruits and seeds. This pigment is a hydrocarbon with the formula $C_{40}H_{56}$. In recent years the study of this compound has received new impetus because of its relation to vitamin A. Several isomers of carotene are known to exist and the probable structural formula of at least one of these (β carotene) has been determined. This isomer of carotene is apparently the most abundant one in leaves. Some authorities consider the molecular formula of vitamin A to be $C_{20}H_{29}OH$ and that this compound is produced in the animal body by the hydrolysis of carotene according to the following equation:

$$C_{40}H_{56} + 2\,H_2O \rightarrow 2\,C_{20}H_{29}OH$$

Lycopene, a red pigment found in the fruits of tomato, red peppers, roses, and other species, and in some flowers, is isomeric with carotene, but apparently has a very different structural formula.

It was formerly supposed that *xanthophyll* was a single compound but it is now considered that there is a group of these compounds, similar but not identical in chemical formula, so it is more accurate to speak of the *xanthophylls. Lutein* seems to be the principal leaf xanthophyll, often constituting half or more of the xanthophyll extracted from leaves. Another common leaf xanthophyll is *zeaxanthin* which, however, is usually present only in relatively small amounts. The molecular formula of both of these xanthophylls is $C_{40}H_{56}O_2$. Chemically the xanthophylls are closely related to carotene and undoubtedly are derived from this compound by various types of chemical transformations.

Carotene and the xanthophylls can both be extracted quantitatively from green leaves by the use of suitable solvents. Neither pigment is soluble in water. Carotene is soluble in ether, chloroform, and carbon bisulfide. The xanthophylls will dissolve in chloroform and alcohol, but are only slightly soluble in carbon bisulfide, and insoluble in petroleum ether.

As shown by its absorption spectrum (Fig. 82), carotene β absorbs only wave lengths in the blue-violet portion of the spectrum. The absorption spectra of other carotinoids are very similar to that of carotene.

Most of the carotinoid pigments are readily synthesized by plants in the

complete absence of light. While various opinions have been expressed regarding the role which these pigments play in photosynthesis as yet no convincing evidence has been discovered that they play any part at all.

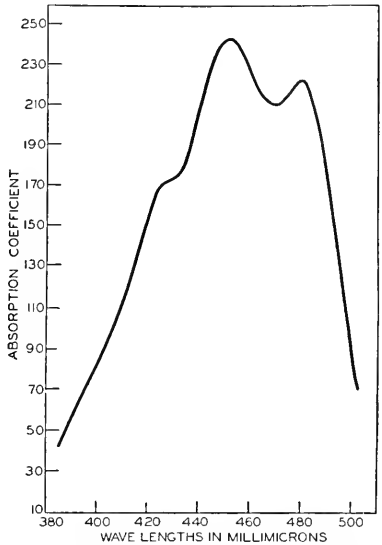

FIG. 82. Absorption spectrum of β-carotene in ether-alcohol solution. Data of Miller *et al.* (1935).

Relative Quantities of the Chloroplast Pigments Present in Green Leaves.—The chlorophylls are present in green leaves in greater quantity than the carotinoids. This fact is illustrated by the data in Table 28 which shows the absolute quantities of each of the four chloroplast pigments in the leaves of the European elder.

TABLE 28—QUANTITY OF EACH CHLOROPLAST PIGMENT IN LEAVES OF THE EUROPEAN ELDER (*Sambucus nigra*) IN JULY EXPRESSED IN GRAMS PER KILOGRAM OF FRESH LEAVES (DATA OF WILLSTÄTTER AND STOLL, 1918).

	Chlorophyll *a*	Chlorophyll *b*	Carotene	Xanthophylls
Sample 1	1.615	0.599	0.146	0.263
Sample 2	1.536	0.552	0.134	0.226
Sample 3	1.732	0.605	0.146	0.301

Of greater significance from a biological standpoint are the *molecular proportions* of these several pigments in the leaves. Since the molecules of

the chlorophylls are heavier than those of the carotinoids, the relative number of molecules of each of these pigments present is not indicated by the data in Table 28. Certain data illustrating this point are presented in Table 29.

TABLE 29—MOLECULAR PROPORTIONS OF THE CHLOROPLAST PIGMENTS PRESENT IN LEAVES AT DIFFERENT SEASONS (DATA OF WILLSTÄTTER AND STOLL, 1918).

Species	Date	Appearance of leaves	Molecular proportions		
			Chlorophyll *a* / Chlorophyll *b*	Carotene / Xanthophylls	Chlorophyll *a* and *b* / Carotene and Xanthophylls
European Elder (*Sambucus nigra*)	July	Green	2.74	0.59	3.33
	Oct. 21	Green	2.61	0.60	3.15
Sunflower (*Helianthus annuus*)	Oct. 7	Green	2.86	0.54	3.66
	Nov. 5	Yellow green	4.01	0.26	1.27
	Nov. 5	Yellow	4.95	0.17	0.32
Horse Chestnut (*Aesculus hippocastanum*)	Oct. 24	Green	4.23	0.47	3.06
	Oct. 24	Yellow green	4.23	0.20	0.81

As shown in Table 29 green leaves usually contain about three molecules of the chlorophylls to every one molecule of the carotenoids, about three molecules of chlorophyll *a* for every molecule of chlorophyll *b*, and about two molecules of xanthophylls for every molecule of carotene. In autumn the carotinoid pigments become relatively more abundant due to the gradual disappearance of the chlorophylls and the proportion of xanthophylls to carotene in the leaves also increases.

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CHAPTER XX

PHOTOSYNTHESIS

The dry matter content of any plant tissue can be determined with a fair degree of accuracy by drying a sample of that tissue in a suitable oven at a temperature of 100° C. The residue remaining after evaporation of the water represents the non-aqueous constituents of the tissue. The percentage dry matter content of plant tissues varies greatly, ranging from 90 per cent or even more in dormant structures such as seeds to 5 per cent or sometimes less in very succulent tissues. That the dry matter fraction of any plant tissue is composed principally of organic compounds can be demonstrated by subjecting it to combustion. This is accomplished by transferring a sample of the dry matter to a crucible and heating it over a flame or in a muffle furnace at a temperature of about 600° C. The small grayish residue resulting from this treatment is called the *ash,* and represents roughly the mineral salts which have been absorbed from the soil (Chap. XXIV). Almost all of the dry matter is oxidized at this temperature and the decomposition products pass off in the form of gases. Practically all of the dry matter which disappears during combustion represents organic compounds which are decomposed as a result of subjection to high temperatures. The ash content of plant tissues varies considerably, but usually lies within the range of 1 to 15 per cent of the dry weight of the tissue. The origin of the organic substance of plants long remained an unsolved problem, but it has now been known for many years that it is synthesized by the plant from a limited number of simple compounds which are absorbed from the soil and the atmosphere.

Photosynthesis as a Process.—The process in which simple carbohydrates are synthesized from carbon dioxide and water by the chloroplasts of living plant cells in the presence of light, oxygen being a by-product, is generally called *photosynthesis.* On the continent of Europe and to a lesser extent in Great Britain, the terms *carbon assimilation* and *assimilation* are often used to designate this process. The common use of the word "assimilation" to denote the process in which foods are incorporated into the structures of the plant body (Chap. XXXI) makes the employment of this term or carbon assimilation as a synonym for photosynthesis undesirable.

The chemical equation representing the process of photosynthesis may be written as follows:

$$6\,CO_2 + 6\,H_2O + 673\text{ kg.-cal.}\ (\text{of radiant energy}) \rightarrow C_6H_{12}O_6 + 6\,O_2$$

As shown in this equation six molecules each of water and carbon dioxide combine in the production of each molecule of hexose sugar, six molecules of oxygen being released as a by-product. For each mol of hexose synthesized radiant energy equivalent to 673 kg.-cal.[1] is converted into the chemical energy of sugar molecules.

It is inconceivable that such a complex chemical process as photosynthesis could take place in one simple step as this equation would seem to indicate. Possible intermediate steps in the process will be considered later. This equation is to be regarded as only a convenient summary statement in the shorthand of chemical symbols.

Leaf Anatomy in Relation to Photosynthesis.—In the vascular plants photosynthesis occurs chiefly in the leaves, which in the majority of species are thin, expanded organs possessing a large external surface in proportion to their volume. This type of structure permits the display of a large number of chloroplast-containing cells to light in proportion to the volume of the leaf. The labyrinth of intercellular air passages in the interior of the leaf is so extensive that practically every green cell is in contact with the internal atmosphere of the leaf. As a result of this loose cellular structure the internal leaf surface (surface of the leaf cells in contact with the intercellular spaces) is much greater than the surface of the epidermal cells exposed to the outside atmosphere. In a lilac leaf, for example, the internal surface is about thirteen times as great as its external surface (Turrell, 1936). Most of the carbon dioxide absorbed by the mesophyll cells diffuses into the cells from the intercellular spaces rather than directly from the outside atmosphere. The presence of intercellular spaces in a leaf therefore provides a much more extensive carbon dioxide absorbing surface than if this gas were absorbed directly through the external leaf surfaces. Since the walls of all of the cells within a leaf are normally more or less saturated with water the vapor pressure of the internal air spaces is usually higher than that of the outside atmosphere. This makes it possible for the leaf cells to absorb atmospheric carbon dioxide without being exposed to the usually relatively dry external atmosphere. Whenever the stomates are open the internal atmosphere of the intercellular

[1] This is the quantity of energy required if glucose is the only sugar synthesized. As the later discussion shows, it is not known with certainty what the first sugar of photosynthesis is. Approximately the same quantity of energy is required, however, for the synthesis of any of the hexoses.

spaces is continuous with that of the outside atmosphere, and carbon dioxide can diffuse with little impediment from the outside air into the intercellular spaces.

The Products of Photosynthesis.—The products of photosynthesis are simple carbohydrates and oxygen. Most of the latter diffuses out of the cells in which it is liberated and plays no further part in the metabolism of the plant. Some of the oxygen, however, may be utilized in respiration within the plant. The products of importance in plant nutrition are the energy-rich carbohydrates which are built up in the chloroplasts.

Sachs termed starch the "first visible product" of photosynthesis, but it has long been recognized that the first substances to appear in living green cells during this process are certain sugars, and that the starch is produced as a result of a secondary and entirely independent reaction.

The three sugars almost universally found in leaf cells during or immediately after photosynthesis are the hexoses: *d*-glucose and *d*-fructose, and the disaccharide: sucrose. Numerous attempts have been made to determine which of these sugars is the first to be produced in photosynthesis. Analyses of the leaves of a number of species of plants have shown that the quantity of hexose remains fairly constant throughout the day, while the amount of sucrose present increases during periods of active photosynthesis, and decreases rapidly upon cessation of photosynthesis (Parkin, 1912, and others). This has been interpreted by some investigators to signify that sucrose is the first sugar produced in photosynthesis. The relatively small fluctuation in the hexose content of leaves may, however, be explained upon the probably more reasonable assumption that all of the hexoses present above a certain concentration are rapidly converted into sucrose or starch (Priestley, 1924).

This latter interpretation is supported by the work of Weevers (1924) who showed that the non-green portions of variegated leaves usually contain only sucrose, while the green, photosynthesizing portions contain both sucrose and hexoses. The same investigator also showed that if geranium plants, the leaves of which had been depleted of sugars by keeping them in the dark, were allowed to photosynthesize for a very short period, only hexoses accumulated. A longer exposure was required for the appearance of sucrose, and a still longer one for the appearance of starch.

As the foregoing discussion indicates it seems reasonably clear that hexoses are the first sugars produced in photosynthesis. A further problem remains, however; which of the hexoses, glucose or fructose, is the first product? No positive answer can be given to this question. It seems probable that either both glucose and fructose are synthesized in photosynthesis, or that a very active form of one of these sugars is produced which can be readily trans-

formed into the other. Starch may then be built up from glucose as described later in this chapter, and sucrose synthesized from glucose and fructose, probably under the influence of the enzyme *sucrase* (Chap. XXVII), according to the following equation:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6} \xrightarrow{\text{Sucrase}} \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O$$

Both starch and sucrose are temporary storage products in the mesophyll cells. The proportions of these two compounds built up from the simple sugars depend upon the species and the intracellular metabolic conditions. A number of species do not synthesize starch in the leaves but sucrose seems to be of universal occurrence in green plants.

The Photosynthetic Ratio.—For many years it has been known that the ratio of the volume of oxygen released to the volume of carbon dioxide absorbed during photosynthesis is approximately one. This ratio is called the *photosynthetic ratio* $\left(\frac{O_2}{CO_2}\right)$.[2] Precise determinations of the photosynthetic ratio are difficult to make, principally because of the simultaneous occurrence of respiration in all living cells. The gaseous exchanges accompanying respiration—consumption of oxygen and release of carbon dioxide—may complicate attempts to measure photosynthetic ratios, particularly since the value of the respiratory ratio is not necessarily constant, even for the same tissue. Nevertheless it is of considerable theoretical importance that exact data regarding the photosynthetic ratio be obtained. Knowledge of the magnitude of this ratio permits certain deductions to be made regarding the nature of the products of photosynthesis. If the primary product of photosynthesis is a fat, a protein, or some other non-carbohydrate, the photosynthetic ratio would not be one. Experimental verification of the theoretical value of one for the photosynthetic ratio will therefore help to validate the generally accepted equation for photosynthesis.

Of the numerous attempts to determine the photosynthetic ratio of plants with precision the best is probably that of Willstätter and Stoll (1918). They made determinations of the photosynthetic ratio under an experimental arrangement such that the rate of photosynthesis was 20 to 30 times as great as the rate of respiration. Under these conditions any slight errors introduced by the gaseous exchanges accompanying respiration are relatively small.

[2] This ratio is sometimes written $\frac{CO_2}{O_2}$ but since this form is universally used for the respiratory ratio (Chap. XXIX) it seems better to adopt the form $\frac{O_2}{CO_2}$ for the photosynthetic ratio.

Measurements upon a number of species of plants within the temperature range 10-35° C., while subjected to such conditions, resulted in values for the photosynthetic ratio which seldom varied by more than ± 0.02 from unity.

Starch Synthesis.—That starch is actually synthesized in the chloroplasts can easily be demonstrated. A potted plant of suitable species is kept in a dark room until the mesophyll cells are starch free. Upon transference to bright light the appearance of starch can be detected in the leaves by means of the familiar iodine test within a relatively short time, in many species in less than an hour. Microscopic examination of the leaves will show that the starch grains are located within the chloroplasts.

However, the presence of starch in the cells of a tissue is not necessarily proof that photosynthesis has taken place in those cells. Starch is insoluble in water and cannot diffuse from cell to cell. The presence of starch grains in a cell must mean, therefore, that the grains have been formed in that cell. Starch grains often occur abundantly in the cells of non-green tissues or in the tissues of roots or other plant organs which are never exposed to light. Obviously photosynthesis cannot take place in such cells and the starch must have been synthesized from sugars translocated to these cells which ultimately came from the green parts of the plant.

Photosynthesis and starch synthesis are therefore two distinct processes. In the higher plants the former occurs only in the chloroplasts and in the presence of light; the latter may occur in the chloroplasts, but also takes place in many non-green cells and in the complete absence of light, providing there is a suitable concentration of sugar in the cells, and other necessary internal physiological conditions prevail. In non-green cells starch is synthesized in the leucoplasts.

The synthesis of starch from glucose may be represented by the following equation:

$$\underset{\text{Glucose}}{n\ C_6H_{12}O_6} \rightarrow (C_6H_{10}O_5)_n + n\ H_2O$$

As this equation shows one molecule of starch is built up from a large number (n) of *d*-glucose molecules, with the elimination of n molecules of water. The exact magnitude of n is unknown. Reactions of this type in which a relatively large number of molecules are combined in the synthesis of one larger molecule with the elimination of water are called *condensation* reactions and are of common occurrence in living organisms.

The starch formed in the chloroplasts of green plant organs is therefore the product of a secondary reaction—*starch synthesis*—which can proceed only when glucose is present in the cell. The critical concentration of simple

sugars required for starch formation in the leaves of many species is very low, and has been reported to be less than 0.5 g. per 100 g. of fresh leaves in some species. In the mesophyll cells of most species, therefore, starch formation in the leaves quickly follows photosynthesis, much of the sugar produced in the latter process being converted into starch. Under conditions favorable to photosynthesis the starch content of the leaves of most species usually increases during much of the daylight period. During the night hours the starch content of leaves usually decreases due to digestion of part or all of the starch back to glucose and translocation out of the cells in the form of this sugar or other soluble carbohydrates produced therefrom.

That starch synthesis is an entirely independent process from photosynthesis is also indicated by the fact that this process does not occur in the mesophyll cells of a number of species of plants, yet photosynthesis takes place in these cells in the same manner as in all other green plants. Failure of the leaves to synthesize starch is a characteristic feature of the metabolism of many species of the *Liliaceae, Amaryllidaceae, Gentianaceae, Compositae,* and *Umbelliferae.*

Similarly the non-green portions of at least some kinds of variegated leaves do not normally synthesize starch. However, if the sugar concentration within the chlorophyll-free cells is artificially increased by floating such leaves on glucose solutions, starch synthesis can be induced. For the leaves of the variegated geranium a glucose solution of about 0.5 M concentration has been found to be optimum for the induction of starch synthesis in the non-green portions (Chapman and Camp, 1932).

The Measurement of Photosynthesis.—Three methods are in common use for measuring the rate of photosynthesis: (1) determination of the rate of oxygen release, (2) determination of the rate of carbon dioxide consumption, and (3) determination of the rate of increase in the dry weight of photosynthetically active organs.

Measurements of photosynthesis are complicated by the fact that certain other processes involving the same materials are proceeding in the cells at the same time. The process of respiration is continually in progress in all cells, resulting in an oxidation of part of the carbohydrates synthesized in photosynthesis. This introduces an error which is inherent in all methods of measuring photosynthesis. Determinations of the quantity of photosynthate produced in a given time are always less than the true value by the amount of carbohydrate which has been consumed in respiration. In many measurements of photosynthesis the simultaneous occurrence of respiration is disregarded, and the results obtained are designated as the *apparent* photosynthetic rate, or, in other words, as the rate of photosynthesis minus the rate of

respiration. Since in rapidly photosynthesizing tissues the rate of photosynthesis is often ten to twenty times as great as the rate of the respiration, the apparent photosynthetic rate is often not greatly less than the true rate. In some experiments upon photosynthetic rates, the values obtained are corrected by adding to them values supposed to represent the quantity of carbohydrates consumed in respiration during the period of the determination. The values used for such corrections are obtained by measuring the respiration rates of the same plant or organ when so treated that photosynthesis cannot occur, as for example after transference to a dark room.

Quantitative measurements of photosynthesis in terrestrial plants, based on determinations of the rate of oxygen evolution or the rate of carbon dioxide consumption are made with the plants or excised leaves enclosed in glass chambers. In most of the more recent applications of this method outside air is passed through the chambers at a relatively rapid rate. The air is collected upon emergence from the chamber and analyzed for oxygen, or carbon dioxide, or both. Another procedure is to analyze small samples of the air for these gases at frequent intervals. By a comparison of the results of these analyses with similar analyses of the outside atmosphere the rate of carbon dioxide consumption or oxygen evolution can be computed, either of which will serve as a measure of the rate of photosynthesis. Investigations in which photosynthesis was measured by this method are described in the next chapter.

A commonly used simple method of measuring the rate of photosynthesis in water plants by the rate of evolution of oxygen is the so-called "bubble-counting" method. When cut shoots of water plants such as the waterweed (*Elodea canadensis*) are illuminated, bubbles may be observed to rise in more or less rapid succession from the cut ends of the stems. Bubbles released from *Elodea* stems usually contain from 30 to 60 per cent oxygen. The greater the rate of photosynthesis, the more rapid the rate of bubble emission. If suitable precautions are taken to minimize sources of error the number of bubbles appearing in a unit time may be taken as a roughly quantitative measure of the rate of photosynthesis. The method will not yield exact results but is satisfactory for demonstrating the general effect of certain external factors upon photosynthesis. The principal sources of error in this method are: (1) the bubbles may vary considerably in size, (2) the oxygen content of the bubbles is not constant but increases as the rate of emission, (3) a part of the oxygen liberated diffuses directly into the surrounding water and thus escapes detection, and (4) movement of the plant or agitation of the circumambient liquid may have a marked effect upon the rate of bubble emission (Wilmott, 1921).

The simplest method of making rough quantitative measurements of the rate of photosynthesis in terrestrial plants is to determine the increase in the dry weight of leaves during a given interval of time. One method of procedure is as follows: A representative sample of disks, usually 100 or more, is cut from the leaves by means of a cork borer at the beginning of the period for which the determination is to be made. These disks are transferred to an oven and dried to constant weight. At the end of a chosen period of time, the plant having been meanwhile exposed to the desired environmental conditions, a second sample consisting of the same number of disks as the first is cut from the leaves. Various precautions must be taken to insure the two samples being as nearly comparable as possible. The dry weight of the second sample of disks is also determined. Any gain in dry weight is considered to represent carbohydrates which have accumulated in the leaves as a result of photosynthesis.

This method is subject to three principal errors: (1) the consumption of a portion of the photosynthate in respiration, (2) translocation of some of the products of photosynthesis out of the leaves, and (3) changes in the area of the leaves (Thoday, 1909). The last error is due to variations in leaf turgidity and is more marked on clear, sunny days. On such a day the area of a leaf shrinks during the daylight hours. Hence, a disk cut out of a leaf in the afternoon will include more cells than one of equal area removed from the leaf in the early morning. Even if no photosynthesis occurs, the former disk will possess a greater dry weight than the latter because it will include more cell wall and protoplasmic material. A part of the gain in the weight of leaves indicated by this method is often due, therefore, simply to changes in the area of the leaves. Cutting out disks of the leaf tissue may also induce metabolic changes in the residual tissue which will influence its rate of photosynthesis. The gain in dry weight of leaves under conditions favorable for photosynthesis is usually between 0.5 and 2.0 g. per square meter per hour.

The "twin leaf" method described by Denny (1930) is a useful variation of this method. This procedure can be followed only with species bearing similar opposite leaves or leaflets. One leaf of a number of pairs is used for the first determination of dry weight, the mates being taken for a similar determination at the end of the experimental period. The accuracy of this method will depend in part upon the number of leaves used in the matched samples. Denny records that when 25 leaves of *Salvia splendens* were used, the error due to a difference in the initial weight of the leaf samples was only about 1 per cent.

The Mechanism of Photosynthesis.—Certain topics contributing to an understanding of the chemical kinetics of photosynthesis have already been considered: (1) the structure of the chloroplasts, (2) the general properties of the chloroplast pigments, (3) the first sugar of photosynthesis, and (4) the photosynthetic ratio. Other aspects of this problem will now be discussed under appropriate sub-headings.

1. *The Rôle of the Chloroplast Pigments.*—Although it has been known for a long time that chlorophyll is essential for photosynthesis the mechanism of its operation in the process is still largely a mystery. There have been two general ideas regarding its rôle: (1) that chlorophyll combines chemically with carbon dioxide or some other compound and takes part in the chemical reactions involved in photosynthesis; and (2) that chlorophyll absorbs certain wave lengths of radiant energy and either converts this energy into other wave lengths that are utilized in photosynthesis, or else in some way transfers the energy absorbed directly to the compounds involved in the reaction.

Determinations of the quantity of chlorophyll present in leaves before and after active photosynthesis show that their chlorophyll content does not diminish during the process. There is not only just as much chlorophyll after active photosynthesis as before, but the proportion of chlorophyll *a* to chlorophyll *b* is the same after a period of active photosynthesis as before. These facts make improbable any theory that photosynthesis is associated with the continuous destruction or transformation of chlorophyll. There is, however, some experimental evidence for the view that chlorophyll enters into a temporary chemical union with carbon dioxide or carbonic acid and that it is returned to its original condition when such groups are split off. Other investigators, however, doubt that even a temporary combination occurs between carbon dioxide and chlorophyll during photosynthesis. The fact that carbonic acid does not absorb any of the wave lengths of the visible spectrum has led to the suggestion that chlorophyll may operate by converting visible radiations into wave lengths which are effective in the reduction of carbonic acid.

The universal presence of the carotinoid pigments in the chloroplasts suggests that they may participate in the process of photosynthesis, but their rôle, if any, is unknown. Chemical analyses of green leaves, made before and after active photosynthesis, do show that both the absolute and the relative amounts of the carotinoid pigments are changed during the process (Table 30). The amount of carotene present decreases and the amount of xanthophylls increases. However, since the conditions prevailing in such experiments favor the oxidation of these pigments, it is doubtful if any conclusions regarding the rôle of the carotinoids in photosynthesis can be drawn from them.

TABLE 30—THE EFFECT OF PHOTOSYNTHESIS UPON THE ABSOLUTE AND RELATIVE QUANTITIES OF THE CHLOROPLAST PIGMENTS IN LEAVES OF CHERRY LAUREL (*Prunus laurocerasus*) (DATA OF WILLSTÄTTER AND STOLL, 1918).

Duration of illumination	Content in mg. per 10 g. of fresh leaves				Ratio of		
	Chlorophyll *a*	Chlorophyll *b*	Carotene	Xanthophylls	Chlorophyll *a* to Chlorophyll *b*	Carotene to Xanthophylls	Chlorophyll *a* + *b* to Carotene + Xanthophylls
Unilluminated	7.2	2.2	0.87	1.65	3.3	0.56	2.3
22 hours.....	7.1	2.4	0.63	2.29	3.1	0.29	2.0

2. *Other Protoplasmic Factors.*—The chloroplast pigments are not the only constituents of the living cell system which are essential for photosynthesis. Although chlorophyll is indispensable in the process it has never been possible to accomplish photosynthesis *in vitro* by the use of chlorophyll solutions or dispersions. There is some evidence that photosynthesis will not occur in the complete absence of oxygen, suggesting that the respiratory system of the cell plays a part in the process. Apparently, however, other systems than the respiratory mechanism are also involved since photosynthesis ceases in most plants at temperatures between 40 and 50° C. and before respiration is completely inhibited. Furthermore, photosynthesis is inhibited by smaller doses of narcotics than respiration. Since neither temperatures up to 50° C. nor narcotics in small doses are known to exert any effect upon the chloroplast pigments these facts indicate very strongly that certain protoplasmic factors other than the pigment system or the respiratory mechanism are essential for the occurrence of photosynthesis. Just how many such protoplasmic factors operate as part of the photosynthetic system it is impossible to say, but considerable evidence indicates that at least one enzymatic mechanism is involved in the process.

3. *Steps in the Photosynthetic Process.*—The experimental evidence at present available indicates with a fair degree of certainty that there are probably at least four distinct steps in the process of photosynthesis: (1) a diffusion phase in which dissolved carbon dioxide or carbonic acid molecules migrate from the cell walls to the chloroplasts, (2) at least one strictly chemical reaction, (3) a photochemical reaction, and (4) at least one reaction catalyzed by an enzyme. This last reaction may be identical with either (2) or (3).

There can be no question about the occurrence of the first of these steps

in the photosynthetic process. After passing into solution in the water of a mesophyll cell wall part of the carbon dioxide reacts with water forming carbonic acid (H_2CO_3) and diffuses to the chloroplasts as this compound. Some of the carbon dioxide, however, diffuses to the chloroplasts in true solution.

Over a temperature range of about 10-25° C., if light intensity and carbon dioxide concentration are relatively high, the temperature coefficient (Chap. VII) of photosynthesis is approximately two. Strictly chemical reactions characteristically have a temperature coefficient of from two to three. This fact indicates that at least one of the reactions involved in photosynthesis is of a purely chemical type. Since this fact was first pointed out by Blackman, this reaction is often called the *Blackman reaction.* It is also frequently referred to as the *dark reaction,* since it does not require light, and therefore may take place in either the light or the dark. It is possible that more than one such "dark reaction" may be involved in photosynthesis.

A chemical reaction which proceeds only at the expense of absorbed light is called a photochemical reaction. That photosynthesis involves such a reaction can be inferred from the fact that it occurs only in the light. The temperature coefficient of photochemical reactions is approximately one. Under low light intensities, even with a relatively high carbon dioxide concentration and other conditions favorable for photosynthesis, the temperature coefficient of the process is about one, indicating that under such conditions the rate of photosynthesis is limited by its photochemical phase.

That photosynthesis involves both a photochemical and a chemical reaction is also shown by the results of investigations in which plants are exposed to intermittent light. The most recent experiments of this type have been performed by Emerson and Arnold (1932). When cultures of *Chlorella* (an alga) were exposed to the intermittent illumination at the rate of 50 flashes per second, the periods of illumination being much shorter (0.0034 sec.) than the intervening dark periods (0.0166 sec.) the photosynthetic yield *per unit of light* was increased about 400 per cent as compared with the rate in continuous light.

Assuming, as most evidence indicates, that the photochemical reaction comes first, the results just described can be explained as follows. When illumination is continuous the products of the light reaction are formed faster than they can be utilized in the relatively slower dark reaction. When the light is intermittent, however, all or most of the products of the photochemical reaction are removed by the dark reaction during the intervening dark period, and the photosynthetic output per unit of light is considerably greater.

By experimenting to find out how long a dark period was required for

the best yield per unit of light, *i.e.* for the complete removal of the product resulting during each light flash, the same investigators were able to estimate the duration of each reaction. The dark reaction was found to proceed in less than 0.04 sec. at 25° C., and to be greatly influenced by temperature. The light reaction, on the other hand, takes place with great speed, requiring only about 0.00001 sec. for its completion, and is unaffected by temperature. The light reaction is affected by the carbon dioxide concentration while the dark reaction is not, indicating that the carbon dioxide enters into the photosynthetic reaction either before or coincident with the photochemical reaction, probably the former. The experimental results of Craig and Trelease (1937) and Pratt and Trelease (1938) indicate that water is involved in at least one of the dark reactions of photosynthesis.

These results indicate that the rate of photosynthesis is limited by the stage of the process which occurs at the slowest rate. At low light intensities and adequate carbon dioxide supply the photochemical reaction is limiting, and temperature will have little effect on the rate of the process. At high light intensities and adequate carbon dioxide supply but low temperatures the rate of photosynthesis is limited by a dark reaction, and will increase considerably with a rise in temperature.

The temperature coefficient of photosynthesis decreases rapidly at temperatures above approximately 35° C. This is in accordance with the behavior of enzymatic reactions generally and suggests that enzymes are involved in the process.

The results of experiments by Molisch (1925) upon the continued release of oxygen by desiccated leaves are also considered to support the concept that enzymes are involved in photosynthesis. Leaves were killed by drying for several days at a temperature of 30-35° C., powdered, ground in water and suspended in a culture of luminescent bacteria. Such bacteria glow only in the presence of oxygen. As soon as the oxygen in such a suspension has been exhausted the luminescence of the bacteria disappears. If the mixture is then illuminated for a brief period the culture begins to glow showing that oxygen is being liberated. Very small concentrations of oxygen are sufficient to produce luminescence so that these bacteria provide a very delicate test for oxygen, probably the most sensitive known. Leaves killed by freezing behaved similarly to the dried leaves but leaves killed by rapid heating or by ether failed to release oxygen. These results seem to indicate that one phase of the photosynthetic process can still continue after such drastic treatments. Furthermore, since after treatments which destroy enzymes (heating, etc.) the leaves lose this capacity, it is believed that this phase of the photosynthetic process is enzymatic in nature. Obviously these experiments do not prove that

the entire complex of chemical, enzymatic, and photochemical reactions involved in photosynthesis can occur in dried leaves as well as in living cells.

4. *The Formaldehyde Theory.*—In 1870 the German chemist, Baeyer, published an hypothesis of photosynthesis which has stimulated study and investigation ever since. Baeyer suggested that the carbon dioxide of the air combined with water forming formaldehyde (CH_2O) with the elimination of oxygen (O_2). This hypothesis is still favorably regarded by many investigators and many attempts have been made to test its validity. Three principal lines of investigation have been followed: (1) attempts to detect formaldehyde in living green cells during photosynthesis, (2) attempts to demonstrate the synthesis of carbohydrates in plants immersed in dilute solutions of formaldehyde, and (3) attempts to duplicate the synthesis of formaldehyde from carbon dioxide and water *in vitro*.

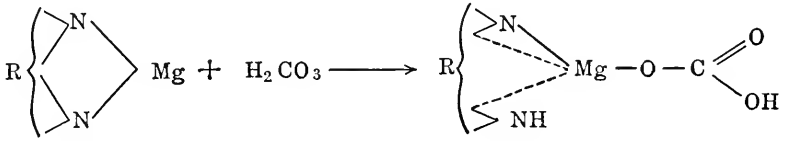
Klein and Werner (1926) tested the leaves of a number of species of plants during active photosynthesis for formaldehyde with dimedon, a compound which rapidly combines with formaldehyde, yielding readily identifiable crystals of the compound formaldomedon. Positive tests were obtained for formaldehyde which was present only in very small quantities (0.008–0.015 g. per 10 g. fresh leaves). This indicates that if formaldehyde is an intermediate product of photosynthesis it must be very rapidly condensed to sugars. In such low concentrations formaldehyde probably would have no toxic effect upon living cells. Klein and Werner reported that they could obtain no tests for formaldehyde in leaves in the dark, in leaves deprived of carbon dioxide, in macerated leaves, in chlorophyll extracts, or in killed or narcotized leaves. Barton-Wright and Pratt (1930), however, claim that formaldomedon can be produced by a photochemical reaction between carbonic acid and dimedon. Their results are in conflict with those of Klein and Werner, and further work is needed before the results of the latter investigators can be accepted without question.

The results of a number of investigators have seemed to show that green plants can use formaldehyde in very low concentrations in the synthesis of sugars. In a recent critical investigation Paechnatz (1937), however, was unable to confirm these results. Not only was there no evidence that any of the several species investigated utilized formaldehyde in photosynthesis, but this compound was found to be highly toxic in very dilute concentrations. With *Elodea canadensis,* for example, toxic effects were noted as soon as the proportion of formaldehyde in the solution exceeded about 0.0004 per cent.

That formaldehyde can be polymerized into sugars in the laboratory by certain treatments has been known for many years. In recent years Baly and his coworkers (Baly and Davies, 1927; Baly and Hood, 1929) have

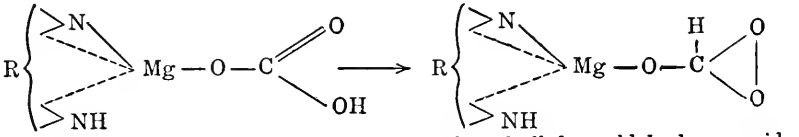
claimed that it is possible to synthesize sugars from carbon dioxide and water under the influence of certain wave lengths of light, an active form of formaldehyde being postulated as an intermediate product. Since several investigators who have tried to duplicate this work have not been able to confirm Baly's results the validity of these conclusions seems in doubt.

5. *Theory of Willstätter and Stoll.*—The theory proposed by these two investigators (1918) may be cited as an example of one of several which attempts to reconcile the formaldehyde theory of photosynthesis with known physiological facts.[3] According to this hypothesis the first step in the process is the combination of a carbonic acid molecule with the magnesium atom of a chlorophyll molecule, forming "chlorophyll-bicarbonate":



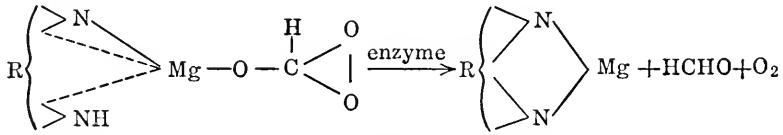
Chlorophyll — Chlorophyll bicarbonate

The next step in the process is supposed to be a photochemical intramolecular shift in the atoms of the chlorophyll-bicarbonate complex resulting in the production of "chlorophyll-formaldehyde peroxide," a compound of higher energy content:



Chlorophyll bicarbonate — Chlorophyll-formaldehyde-peroxide

Following this reaction the chlorophyll-formaldehyde-peroxide is supposed to be split by an enzyme with chlorophyll, formaldehyde, and oxygen as the cleavage products:



Chlorophyll-formaldehyde-peroxide — Chlorophyll

[3] An elaboration of this theory has been proposed by Stoll (1932).

The final stage in the photosynthetic process is presumed to be the polymerization of the formaldehyde resulting in a hexose sugar:

$$6\ HCHO \rightarrow C_6H_{12}O_6$$

Various other theories have been proposed to account for the mechanism of photosynthesis (see for example Emerson and Green 1937, and Franck and Herzfeld 1937), but none of them should be entertained too seriously. They are of value in so far as they present current or recent concepts of the mechanism of the process, but they are subject to more or less continual change, as new facts regarding photosynthesis are discovered.

Magnitude and Efficiency of Photosynthesis.—Transeau's (1926) calculations of the "energy budget" for an acre of corn (maize) plants illustrate very clearly the material and energy relations of photosynthesis. This energy budget was prepared for an hypothetical acre of corn (10,000 plants) growing in north central Illinois and yielding the very good return of 100 bushels per acre. The growing season is assumed to be 100 days. The magnitude of photosynthesis for such a field of corn is shown in Table 31.

TABLE 31—QUANTITY OF PHOTOSYNTHATE PRODUCED BY ONE ACRE OF CORN IN A GROWING SEASON (DATA OF TRANSEAU, 1926)

Dry weight of average corn plant	Grain	216 g.
	Stalk	200
	Leaves	140
	Roots	44
		600 g.
Total dry weight of an acre of corn (10,000 plants) at end of season		6000 kg.
Total ash (5.37 per cent of dry weight)		322 kg.
Total organic matter in the plants		5678 kg.
Total carbon accumulated (44.58 per cent of the organic matter)		2675 kg.
Glucose equivalent of accumulated carbon ($C_6H_{12}O_6 : C_6 = 180 : 72$)		6687 kg.
Glucose equivalent of respired carbon (calculated from estimated rate of CO_2 release = 1 per cent of dry weight per day)		2045 kg.
Total sugar manufactured in terms of glucose		8732 kg.

The quantity of radiant energy falling on an acre of land surface in north central Illinois during the growing period of 100 days is known from measurements made at Madison, Wisconsin, not far from the region of north central Illinois. Using these data together with his own estimates of the total glucose produced by the photosynthetic activity of the corn plants, Transeau was able to calculate the photosynthetic efficiency of corn (Table 32).

TABLE 32—EFFICIENCY OF PHOTOSYNTHESIS IN CORN (DATA OF TRANSEAU, 1926)

Energy required for synthesis of 1 kg. glucose	3760 kg.-cal.
Total energy utilized in photosynthesis by an acre of corn plants in manufacture of 8732 kg. glucose	33 million kg.-cal.
Total solar energy available on the acre during growing season	2043 million kg.-cal.
Per cent of available energy used by corn plant in photosynthesis—*i.e.* its photosynthetic efficiency	1.6 per cent

Of the total carbohydrate synthesized by the corn plant only about 25 per cent is harvested as a grain crop. The hundred bushels of corn grain obtained at the end of the growing season represents only about 0.4 per cent of the total radiant energy which fell on the acre. Most crop plants are considerably less efficient than this unusually productive acre of corn as converters of radiant energy into chemical energy.

The calculation of the efficiency of photosynthesis just presented was based upon the total radiation incident upon the *acre*. This is the correct basis of calculation for evaluations of the efficiency of any plant as a crop. For calculating the efficiency of the process itself other bases of computation are generally used. The efficiency of photosynthesis may be computed on the basis of: (1) the total radiant energy incident upon the leaf, (2) the radiant energy actually absorbed by the leaf, or (3) the radiant energy actually absorbed by the chloroplasts.

Discussion Questions

1. What would be the effect upon plants of the disappearance of all animals from the earth? the effect upon animals of the disappearance of all plants?
2. Is it true that no life could exist upon the earth in the absence of photosynthesis?
3. What are some possible explanations of the failure of leaves of some species to synthesize starch from the photosynthetic products?
4. Under what conditions would you expect that exposure of a plant to intermittent light would not result in an increase in the quantity of photosynthate produced per unit of light?
5. When leaves of some species are submerged in water slow infiltration of the intercellular spaces occurs if they are kept in the dark, but not if they are kept in the light. Explain.
6. Masses of filamentous algae are often found floating near the surface of a pond after several days of clear weather, but the same algal masses are often submerged after a period of several cloudy days. Explain.
7. Most soils contain considerable quantities of organic matter in the form of humus and derivative compounds. How could you demonstrate that green plants do not utilize any appreciable quantity of these compounds as foods?
8. What method would you recommend for measuring the rate of photosynthesis

of a bean plant growing in the field. Point out the possible sources of error in the method.

9. One source of error in the "dry weight" method of measuring photosynthesis is that translocation of foods occurs away from the leaves. Would this error be reduced if the phloem of the petioles could be cut? Would any other errors be introduced?
10. A green plant in a sealed glass vessel containing air enriched with CO_2 was exposed to light of very low intensity for several hours. The air in the chamber was found to contain the same quantity of O_2 at the end as at the beginning of the experiment. Does this indicate that no photosynthesis has taken place?
11. Since only a small percentage of the incident light is utilized in photosynthesis why cannot the light intensity be reduced to this value without retarding the rate of photosynthesis?

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CHAPTER XXI

FACTORS AFFECTING PHOTOSYNTHESIS

The process of photosynthesis is conditioned by a number of different factors, some external, others internal. Four environmental factors are of primary importance in influencing the rate of photosynthesis: (1) light, (2) carbon dioxide concentration of the atmosphere, (3) temperature, and (4) water. Other environmental conditions which can be shown to influence the rate of this process include (1) oxygen, (2) various chemicals, (3) wounding, (4) mineral salt supply, and, in the case of water plants, (5) the osmotic pressure of the aqueous medium. Some of the factors in this latter group rarely if ever have any effect upon photosynthesis under natural conditions, and their influence is only known from laboratory experimentation. The internal conditions influencing the rate of photosynthesis are much less completely understood than the environmental factors. Several such factors have been very definitely recognized, however, as follows: (1) chlorophyll content of the leaves, (2) hydration of the protoplasm, (3) leaf anatomy, (4) protoplasmic factors, including enzymes, and (5) accumulation within the cells of the products of photosynthesis.

The Principle of Limiting Factors.—Earlier investigators of the effects of various conditions upon the rate of photosynthesis attempted to distinguish among *minimum, optimum,* and *maximum* values for each factor in relation to photosynthesis. In evaluating the effect of temperature upon photosynthesis, for example, it was generally considered that there was a minimum temperature below which no photosynthesis occurred, an optimum at which the process takes place most rapidly, and a maximum above which photosynthesis ceases. Advocates of this point of view, however, soon found themselves confronted with the anomalous situation of a fluctuating "optimum." The "optimum" carbon dioxide concentration was found to be greater at high light intensities than at low ones, the "optimum" temperature was found to vary with the light intensity, the "optimum" light intensity was different for plants well supplied with water than for those which were inadequately supplied, etc.

The first important step in the clarification of this problem of the influence of various factors upon photosynthesis was taken when Blackman

(1905) enunciated the "principle of limiting factors." This principle is essentially an elaboration of Liebig's "law of the minimum" (Chap. XXXIII) and was stated by its author as follows: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor."

The explanation of this principle can best be presented in terms of the illustration given by Blackman (Fig. 83). Assume the intensity of light to be just great enough to permit a leaf to utilize 5 cc. of carbon dioxide per hour in photosynthesis. If only 1 cc. of carbon dioxide can enter the leaf in an hour the rate of photosynthesis is limited by the carbon dioxide factor. As the carbon dioxide supply is increased the rate of photosynthesis is also

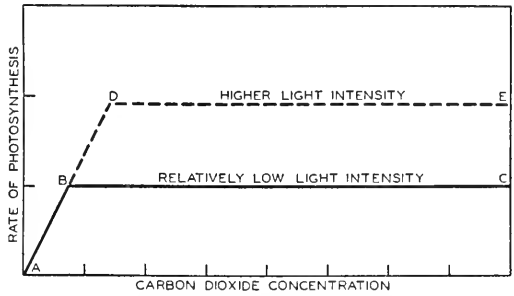

FIG. 83. Diagram to illustrate Blackman's interpretation of the principle of limiting factors.

increased until 5 cc. of carbon dioxide enter the leaf per hour. Any further increase in the supply of carbon dioxide will have no influence upon the rate of photosynthesis, unless a sufficient concentration is present to bring about toxic effects, because insufficient light energy is available to permit its utilization. Light has now become the limiting factor and further increase in the rate of photosynthesis can only be brought about by an increase in the intensity of light. These results are indicated graphically as *A B C* in Fig. 83. This theory assumes a progressive increase in the rate of process with a quantitative increase in the limiting factor (in this example, carbon dioxide) until the point is reached at which some other factor becomes limiting (in this example, light intensity). At this point the increase stops abruptly (point *B* in Fig. 83), and the rate of photosynthesis becomes constant (*B C* of Fig. 83). According to this concept when the magnitude of photosynthesis is limited by one of a set of factors only a shift in that factor towards a condition more favorable for the process will result in an increase in the rate of photosynthesis.

If the light intensity is sufficient to permit the leaf to utilize 10 cc. of carbon dioxide per hour, then the rate of photosynthesis will rise with increase in the carbon dioxide concentration up to a value about twice as great as that at which the maximum rate of photosynthesis was attained at the lower light intensity. The results under these conditions can be indicated graphically by *A D E* (Fig. 83).

Light and carbon dioxide are not the only factors which can be limiting in the photosynthetic process. Theoretically, as examples given later in the chapter will show, any of the factors which influence this process can, under certain conditions, become limiting.

Most subsequent workers (Harder, 1921, James, 1928, and others) have been unable to accept the principle of limiting factors in quite the simple form in which it was first proposed by Blackman. Most investigators have found that when the rate of increase of photosynthesis is plotted along the ordinate with the quantitative variations in some one factor as the abscissa, the resulting curve is not found to show an abrupt transition to the horizontal (points *B* and *D*, Fig. 83) as postulated by Blackman's formulation of this principle, but shows instead a gradual transition to a position approximately parallel to the abscissa. The general type of curve found by most investigators for this relation is shown in Fig. 85. Within this transition region it is evident that increase in either of the two factors involved will result in an increase in the rate of photosynthesis.

The explanation for the occurrence of this gradual transition in the direction of the curve, rather than the abrupt change postulated by the original Blackman theory probably rests principally upon two circumstances. In the first place the seat of the photosynthesis is in the chloroplasts of which there are millions in even a small leaf. Patently it is impossible that each and every chloroplast will be subjected to exactly the same conditions at exactly the same time. All of the chloroplasts are not equally exposed to light, neither are all of them equally well supplied with carbon dioxide. As a factor approaches a limiting value it may check the rate of photosynthesis in some chloroplasts sooner than in others. It is quite possible therefore for light to be the limiting factor for some chloroplasts, while carbon dioxide is simultaneously the limiting factor for other chloroplasts. Similar comments apply to most of the other factors influencing photosynthesis. Hence the rate of photosynthesis as measured in terms of entire organs will exhibit only a gradual change when factors affecting the process are modified, and there exist well defined regions in curves such as those shown in Fig. 85, in which two or even more factors may be considered to act simultaneously as limiting factors.

A second possible explanation of the fact that the rate of photosynthesis shows a gradual rather than an abrupt change with modification in the intensity of factors influencing the process is based upon more theoretical grounds. Certain external factors may not affect the process directly, but influence instead some internal condition which, in turn, affects photosynthesis directly. Within certain ranges of values which show up as "transition zones" on curves plotted as in Fig. 85 increase in either of a pair of external factors may result in an increase in the magnitude of this internal factor, and hence in the rate of photosynthesis. Beyond one limit of the transition range the effect of one of the factors is so predominant that it acts essentially as a limiting factor, while beyond the other limit the other factor acts in a similar manner. Such relationships may actually be more complicated than has been indicated, as it is possible that a single internal factor may often be influenced by more than two external factors.

It should be clearly understood that in speaking of "limiting factors" as applied to physiological processes, that it is not their absolute magnitude which is significant, but their relative magnitude in proportion to the amounts actually required in the process. The quantitatively smallest factor does not necessarily condition the rate of the process, since it may be necessary only in traces, while larger amounts of some other material, or a greater intensity of some other factor may be necessary. To illustrate, suppose that we assume ten units of *a*, two units of *b*, and one unit of *c* are necessary for the formation of one unit of *d*. If we suppose that only five units of *a* are available, none of *d* can be formed regardless of the quantities of *b* and *c* available. Although *c* is an absolute minimum, *a* is in relative minimum, and thus acts as the limiting factor. For this reason many authorities consider it to be more satisfactory to speak of the "relatively limiting factor," "factor in relative minimum," or "most significant factor," rather than of the "limiting factor."

The modifications which have been imposed upon the original concept of limiting factors do not invalidate this principle as a good approximation to the facts, nor destroy its value as a point of view from which to interpret the influence of various factors upon the rate of photosynthesis. Whether the effect of an external factor is direct or indirect, whether the curves break sharply or show a gradual change of direction, the significant fact is that the rate of the process, except in relatively narrow transition regions, is usually largely determined by the least favorable factor, which may for convenience be spoken of either as the limiting factor, or as the factor in relative minimum.

The principle of limiting factors is applicable to all physiological processes and will receive further evaluation in relation to growth phenomena in Chap. XXXIII.

The Rôle of Carbon Dioxide.—All of the carbon dioxide used by green cells in the process of photosynthesis reaches the chloroplasts in solution in water or as carbonic acid which is dissolved in the water. In land plants the atmosphere is the only important source of carbon dioxide. Carbon dioxide released in the process of respiration may be utilized in photosynthesis without leaving the plant, but this seldom constitutes an important part of the total amount consumed. The carbon dioxide utilized in photosynthesis by submerged water plants diffuses into them from the surrounding water.

The atmosphere is composed chiefly of two gases, nitrogen (about 78 per cent) and oxygen (about 21 per cent), but also contains, in addition to a variable but never large amount of water-vapor, small quantities of other gases. One of its minor constituents, carbon dioxide, which constitutes on the average only about 0.03 per cent by volume of the atmosphere, plays a role of the greatest significance in the biological world. As a result of the photosynthetic activity of green plants, the carbon dioxide from the air becomes chemically bound for periods of indefinite length in the organic molecules of living organisms. In view of its important biological rôle the proportion of carbon dioxide in the atmosphere seems precariously small. The actual amount present, however, is enormous. The best estimates, necessarily very approximate, place the total quantity of carbon dioxide in the atmosphere at about 2×10^{15} kilograms. According to the estimates of Schroeder (1919) the quantity of carbon dioxide used annually in photosynthesis by all of the plants on the earth's surface is about 60 million million kilograms.

1. *Sources of the Atmospheric Carbon Dioxide.*—While green plants are continually removing carbon dioxide from the atmosphere, other processes are continually replenishing the atmospheric reservoir with this gas. Carbon dioxide is continually being returned to the atmosphere as a product of the respiration of plants and animals. Contrary to popular opinion plants are undoubtedly more important producers of carbon dioxide than animals. Carbon dioxide is released into the atmosphere as a result of the respiration of both green and non-green plants. The relatively great importance of the latter group of organisms as generators of this gas is not always appreciated. The organic residues of plants and animals are decomposed as a result of the activities of bacteria and fungi. During such decay processes the carbon of these residues is mostly released in the form of carbon dioxide as a result of the metabolic activities of these organisms, and escapes into the air. The evolution of carbon dioxide gas by soils is often very considerable and is frequently referred to as "soil respiration." Most of the carbon dioxide lost from a soil into the atmosphere results from the metabolic activities of soil microorganisms although smaller quantities are released in the respiration of roots

and subterranean species of higher animals. An acre of forest soil may release as much as 20 lb. (9 kg.) of carbon dioxide gas per *hour*. Rates in agricultural or meadow soils are lower, but are nevertheless very appreciable. The respiration of the soil bacteria alone probably results in a greater return of carbon dioxide to the atmosphere than the respiration of all animals.

Carbon dioxide is also released into the atmosphere from volcanos, mineral springs, and in the combustion of coal, oil, gasoline, wood, and other fuel materials. Spoehr (1926) has estimated that the combustion of the world's annual output of coal in 1920—some 1,317,000,000 metric tons—would produce 338×10^{10} kg. of carbon dioxide or only about 0.16 per cent of the existing supply. Such a small annual increment in the carbon dioxide supply could not be detected. On the other hand the weathering of certain igneous rocks (feldspars) combines carbon dioxide and thus tends to reduce the quantity of this gas in the atmosphere.

Oceans and bodies of fresh water are even more important reservoirs of carbon dioxide than the atmosphere. The former are far more important than the latter as storehouses of carbon dioxide. The oceans occupy nearly three-fourths of the earth's surface, and ocean water contains nearly 50 cc. of carbon dioxide including both dissolved and combined forms (carbonates and bicarbonates) per liter. The absolute carbon dioxide content of the oceans is estimated at from thirty to forty times as much as is present in the atmosphere. The carbon dioxide in ocean waters is involved in a complex series of chemical and biological cycles which have never been fully evaluated. Marine plants consume carbon dioxide in photosynthesis and release it in respiration. Marine animals feed either upon marine plants or other animals, but ultimately, as with land animals, all of their food comes from the process of photosynthesis. A part of the carbon in the food consumed by such organisms is released into the water as carbon dioxide in the process of respiration. Aquatic micro-organisms accomplish the decay of dead plants and animals, releasing most of the carbon in these organic remains in the form of carbon dioxide. Complex equilibria between the dissolved carbon dioxide, carbonates, and bicarbonates also exist. Some marine animals precipitate large quantities of carbon dioxide in chemically combined form as the calcium carbonate of calcareous rocks. The conversion of bicarbonates into carbonates results in the release of carbonic acid and thus increases the available carbon dioxide content of the water. Eventually such rocks (limestones, etc.) may be raised above sea level and the carbon dioxide tied up in the form of carbonates again released to the atmosphere or dissolved in running water during dissolution of the rock. Similar although not quite such complex cycles of carbon dioxide exist in the bodies of fresh water.

There is also a constant exchange of carbon dioxide between the oceans and the atmosphere. In fact, on theoretical grounds, there is a good reason to suppose that the carbon dioxide concentration of the atmosphere is more or less effectively maintained in dynamic equilibrium with that of the oceans. Carbon dioxide probably escapes from the oceans whenever its atmospheric concentration falls below the usual value, and dissolves in the oceans whenever a

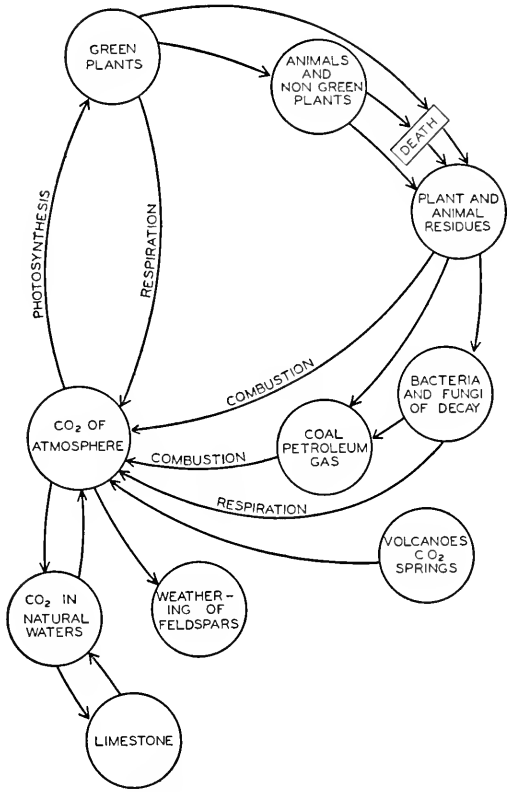

FIG. 84. The carbon cycle.

contrary shift in atmosperic carbon dioxide concentration occurs. The maintenance of such a large-scale dynamic equilibrium between the oceans and the atmosphere is probably the principal factor accounting for the constancy of the carbon dioxide concentration of the atmosphere.

The cycle of carbon in nature is shown diagrammatically in Fig. 84.

2. *The Entrance of Carbon Dioxide.*—Some of the earlier plant physiologists believed that carbon dioxide gas was absorbed by leaves directly through

the epidermis. Critical experiments have shown, however, that the proportion of this gas entering leaves by this route is relatively small, and that practically all of the carbon dioxide entering leaves diffuses in through the stomates.

The rate of entrance of carbon dioxide is very considerable in proportion to the aggregate area of the stomatal pores. Under conditions favorable for photosynthesis Brown and Escombe (1900) found that carbon dioxide would diffuse into a catalpa leaf from the atmosphere at the rate of 0.07 cc. per square centimeter of leaf surface per hour. Since the stomates in this leaf occupy only 0.9 per cent of surface, diffusion of carbon dioxide gas through them took place at a rate of 7.77 cc. per square centimeter of stomatal aperture per hour. Under the same conditions a normal solution of sodium hydroxide absorbs from the atmosphere, even in rapidly moving air, only 0.177 cc. of carbon dioxide per square centimeter per hour. In other words carbon dioxide gas diffuses through the stomates at a rate approximately fifty times as fast as it diffuses into an efficient absorbing surface.

The extremely rapid rate of diffusion of carbon dioxide gas through the stomates can be interpreted in the light of the principles of diffusion of gases through small openings. Table 33, condensed from the work of Brown and Escombe, indicates that these same principles hold, in general, for carbon dioxide as well as water-vapor (Chap. XIII.)

TABLE 33—DIFFUSION OF CARBON DIOXIDE THROUGH MULTIPERFORATE SEPTA. PORES 0.380 MM. IN DIAMETER; SEPTA 1 CM. FROM SODIUM HYDROXIDE SOLUTION (DATA OF BROWN AND ESCOMBE, 1900).

Septum	Area of tube, cm.^{2}	Distance apart of pores in diameters	Diffusion CO_2 through septum, cc. per hour	Diffusion CO_2 through open tube, cc. per hour	Per cent of septum area occupied by pores	Diffusion through pores as per cent of diffusion through open tube
1	9.347	2.63	0.433	0.771	11.34	56.1
2	9.186	5.26	0.401	0.775	2.82	51.7
3	9.456	7.80	0.312	0.768	1.25	40.6
4	9.511	10.52	0.241	0.767	0.70	31.4
5	9.186	13.10	0.156	0.744	0.45	20.9
6	9.347	15.70	0.106	0.740	0.31	14.0

The most important principle illustrated, that the rate of diffusion does not decrease proportionately with reduction in the aggregate area of the pores, is shown in the last two columns. For septum 4 (pores 10.52 diameters

apart), for example, the diffusion is 31.4 per cent of that from the open tube, although only 0.70 per cent of the septum surface is occupied by the apertures.

Brown and Escombe also calculated the *theoretical* carbon dioxide absorbing capacity of a sunflower leaf, using the following as average values: cross-sectional area of a stomatal pore, 0.0000908 mm.^{2}; length of stomatal pore, 0.014 mm.; and number of stomates per square centimeter, 33,000. A zero partial pressure of carbon dioxide in the intercellular spaces was assumed. According to their calculations, when the stomates are fully open carbon dioxide theoretically could diffuse into the leaf at the rate of 2.095 cc. per hour per square centimeter of leaf surface in quiet air. The corresponding figure for rapidly moving air is 2.578 cubic centimeters. As shown by the figures given in the second paragraph under this sub-topic these values are many times greater than any observed values for the rate of diffusion of carbon dioxide into sunflower leaves exposed to the atmosphere. The diffusive capacity of the stomates is clearly more than adequate to account for the necessary rate of entrance of carbon dioxide into leaves even when photosynthesis is occurring at its maximum rate.

3. *Effects of Variations in the Concentration of Atmospheric Carbon Dioxide upon the Rate of Photosynthesis.*—The effect of various concentrations of carbon dioxide upon the rate of photosynthesis of wheat plants exposed to different light intensities is depicted graphically in Fig. 85. In general, with increase in the concentration of atmospheric carbon dioxide, there is an increase in the rate of photosynthesis until some other factor, in this example light intensity, becomes limiting. Furthermore, as long as the limiting effect of another factor does not become apparent, the rate of photosynthesis is approximately proportional to the concentration of carbon dioxide. It should be noted, however, that even the highest light intensity employed in this experiment is very much less than the intensity of full summer sunlight.

The foregoing statements only apply when plants are exposed to relatively low concentrations of carbon dioxide. Higher concentrations of this gas (15–25 percent) exert an inhibitory action upon many plant processes including photosynthesis. The exact concentrations at which this checking effect of carbon dioxide upon photosynthesis becomes apparent in various species does not seem to have been determined. The rate of photosynthesis in practically all species shows an increase with increase in the concentration of carbon dioxide gas up to at least twenty or thirty times the percentage usually present in the atmosphere, if no other factors are limiting. For many species the carbon dioxide concentration can be raised to values considerably in excess of this before any inhibitory effect upon photosynthesis can be detected.

Under natural conditions during the summer months in temperate regions the carbon dioxide concentration of the atmosphere is most frequently the limiting factor in photosynthesis for all photosynthetic tissues which are well exposed to light. Hence if the concentration of this gas in the atmosphere could be increased it would usually result in an increased amount of photosynthesis. Increased photosynthesis in crop plants usually means a greater yield so there are excellent practical reasons for attempting to raise the concentration of carbon dioxide in the atmosphere. Artificially increasing the

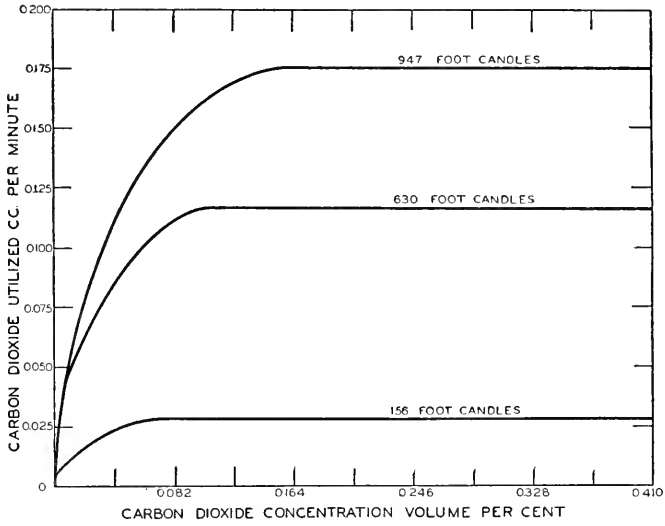

FIG. 85. Relation between different carbon dioxide concentrations and rate of photosynthesis in wheat at three different light intensities. Data of Hoover, *et al.* (1933).

carbon dioxide content of greenhouses during seasons when some other factor is not limiting has been found to result in an improved development of greenhouse crops (Cummings and Jones, 1918.) Increased yields are also said to have been obtained from field crops which were provided with additional carbon dioxide by means of pipes laid on or in the ground. It is doubtful, however, whether this latter practice can ever be developed upon a commercially practical basis.

Investigations of Lundegårdh (1931) and others indicate that a considerable part of the carbon dioxide utilized by plants in many habitats is produced locally by "soil respiration," which represents largely the respiration of micro-organisms. A part of the favorable effect of the application of fertilizers or organic manures on crop production is undoubtedly due to the result-

ing indirect increase in the carbon dioxide content of the lower layers of the atmosphere. Application of fertilizers not only favors increased growth of the higher plants, but also leads to an increased development of soil microorganisms. The increased respiration of these organisms results in measurable increases in the carbon dioxide concentration of the stratum of the air close to the ground level. This local enrichment in the carbon dioxide content of the atmosphere will favor photosynthesis in all low-growing species of plants, and often results in lower leaves or branches on a plant being exposed to considerably higher concentrations of carbon dioxide than other leaves or branches only a few feet higher above the soil level. On well fertilized fields the production of carbon dioxide by soil respiration during a twenty-four hour period may equal or exceed the consumption in photosynthesis during the daylight hours. Over unfertilized fields, pasture lands, etc. the air close to the ground is often relatively low in carbon dioxide concentration, especially during the daylight hours when environmental conditions are favorable to the occurrence of photosynthesis at appreciable rates.

Similarly in forests the carbon dioxide concentration of atmospheric layers close to the soil surface is often several times as great as in layers higher up, due to intensive soil respiration. This favors photosynthesis and growth in herbs, shrubs, and young trees which do not rise far above the floor of the forest. On the other hand, in the atmosphere on a level with the crowns of the trees the carbon dioxide content is sometimes considerably less than the average atmospheric value during the hours when photosynthesis is in progress.

The Rôle of Light.—The energy stored by green plants in the molecules of simple sugars during photosynthesis can be supplied only by light, and in the absence of illumination photosynthesis fails to occur. Any source of radiant energy which furnishes wave lengths within the range of the visible spectrum will induce photosynthesis, provided its intensity is sufficiently great. Although a few of the longer wave lengths of ultraviolet apparently are effective in photosynthesis, in general this process can occur only in radiation of the visible part of the spectrum. Under natural conditions sunlight, either direct or reflected from the sky, other objects, etc. is the only source of radiant energy supplying wave lengths which can be used in photosynthesis. Photosynthesis will occur under electric lights or other artificial sources of illumination if of sufficient intensity. Such light sources are often used in experimental work on photosynthesis and to some extent in greenhouses as supplementary sources of illumination.

Light, like all forms of radiant energy, varies in intensity, quality, and duration (Chap. XIX), and the influence of this factor upon photosynthesis

will be discussed under these three headings. Before the various effects of light upon photosynthesis are considered, however, it will be desirable to analyze the physical relations between leaves and incident light.

1. *The Optical Properties of Leaves.*—Of the visible light which falls on leaves, as with radiant energy generally, a part is reflected, a part is transmitted through the leaf, and a part is absorbed by the leaf. The proportion of the visible light incident upon a leaf which is absorbed varies considerably according to the kind of leaf and the intensity of light but is frequently in the neighborhood of 80 per cent (Seybold, 1932) and probably often higher.

For any given leaf the proportion of the incident light reflected varies considerably according to wave length (Shull, 1929). In all normally green

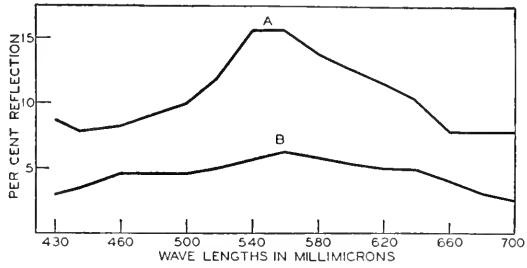

FIG. 86. Per cent reflection of different wave lengths of light from leaves of lilac (*Syringa vulgaris*). (*A*) from lower surface, (*B*) from upper surface. Data of Shull (1929).

leaves, however, maximum reflection apparently occurs in the range of wave lengths corresponding to the green portion of the spectrum (Fig. 86).

Similarly the proportion of incident light of each wave length which is transmitted varies considerably depending upon the kind of leaf. For normally green leaves transmission is relatively high in the green, relatively low in the blue violet and short red, and greatest in the long red (Fig. 87). The green color of leaves is due to the proportionately greater reflection and transmission of light in the green region of the spectrum.

2. *Effects of Variations in the Intensity of Light upon the Rate of Photosynthesis.*—The effect of various light intensities upon the rate of photosynthesis of wheat plants exposed to different atmospheric concentrations of carbon dioxide is depicted graphically in Fig. 88. In general with increase in light intensity there is an increase in the rate of photosynthesis until some other factor, in this example, the carbon dioxide concentration, becomes limiting. At relatively low light intensities, as long as carbon dioxide is not the limit-

ing factor, the rate of photosynthesis is approximately proportional to the light intensity. For the maximum concentration of carbon dioxide indicated in Fig. 88 (0.11 per cent) the maximum light intensity used was not great enough for the carbon dioxide factor to have become limiting. It should also be noted that the maximum light intensity employed—1000 foot candles—is much inferior in intensity to usual summer sunlight, which at noon on a clear day usually is equivalent to 8,000-10,000 foot candles. These results indicate that light is not a limiting factor in photosynthesis when leaves are exposed to full sunlight if the carbon dioxide content is within the range normally found in the atmosphere. Under such conditions maximum photosynthesis per unit of leaf area is attained in most and probably all species at light intensities considerably less than that of full sunlight.

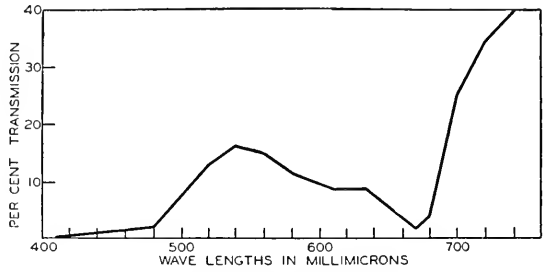

FIG. 87. Per cent transmission of various wave lengths of light by leaves of box elder (*Acer negundo*). Data of Seybold (1933).

Results such as those depicted in Fig. 88 will be obtained only when a single leaf or a small plant, all parts of which are well illuminated, is used as the experimental material. When the effect of light on photosynthesis is considered in terms of an entire tree, however, a different relation holds. Thus Heinicke and Childers (1937) showed that the rate of photosynthesis for an entire apple tree increased progressively with increase in light intensity up to that of full sunlight. This is undoubtedly due to the fact that many of the interior leaves on a large tree are heavily shaded. Although, in general, maximum rates of photosynthesis are attained in the leaves of most species at light intensities considerably below that of full sunlight (in the apple tree at one-fourth to one-third of this value) these investigators showed that many of the interior leaves of an apple tree receive 1 per cent or even less of the sunlight received by peripheral leaves. Even in full sunlight, therefore, many of the leaves on a tree do not photosynthesize at their maximum capacity. The lower the light intensity the greater the proportion of the leaves of which this will be true. Hence the greater the intensity of the

incident light the greater the average rate of photosynthesis per unit of leaf area. The total photosynthesis per tree therefore increases progressively with increased illumination up to the maximum possible sunlight intensity.

The intensity of the sunlight incident on the earth's surface varies from hour to hour and from season to season, as well as with meteorological conditions. Clouds, fogs, dust, atmospheric humidity, etc. all influence the intensity of the radiation which reaches the surface of the earth. The exposure and pitch of a slope are also factors influencing the intensity of the light impinging upon a given location, and are particularly of importance in hilly

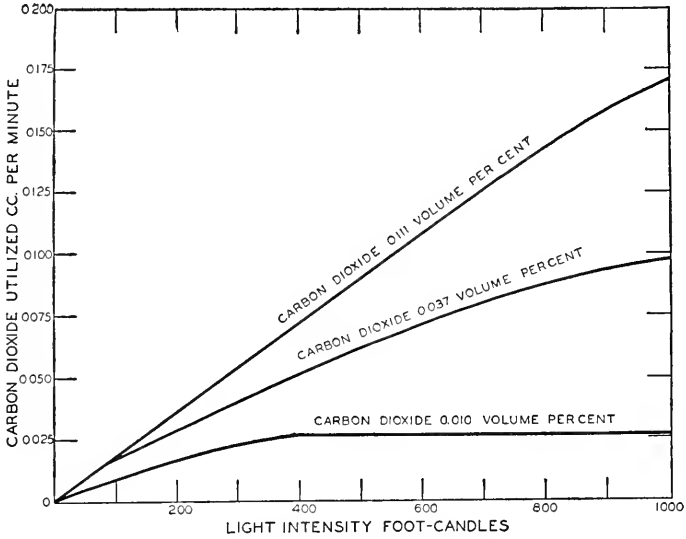

FIG. 88. Relation between different light intensities and rate of photosynthesis of wheat plants at three different carbon dioxide concentrations. Data of Hoover, *et al.* (1933).

or mountainous country. Other conditions being equal the intensity of sunlight also increases with increase in altitude. Most variations in the intensity of natural light are accompanied by variations in light quality of greater or lesser magnitude. Usually, however, under out-of-doors conditions variations in the intensity component of light are of greater physiological influence than the accompanying variations in the quality component.

Ecologically one of the most important factors causing different species of plants to be exposed to differences in light intensity is the effect of taller plants in shading those of lesser stature. Some species will thrive and photosynthesize efficiently only in fully exposed locations, others can complete their normal life cycles in intensely shaded habitats.

Even under a tree with a rather open crown the light intensity is only one-tenth to one-twentieth that of full sunlight. Hence light is usually the limiting factor for photosynthesis in most species of plants when growing in the shade of trees. Species which normally grow in deep shade are probably exceptions to this statement. On cloudy days light is also usually the limiting factor in photosynthesis. Because of the prevalence of cloudy weather in many regions during the winter, especially in December and January, plants under glass often photosynthesize at very low rates during those months. The short length of the daylight period also contributes to low daily rates of photosynthesis during the winter season.

A number of studies have been made of the minimum light intensities at which various species of plants are just barely able to survive. Unless reserve foods have previously accumulated, the minimum light intensity at which a plant will remain alive for indefinite periods during its normally active seasons must permit sufficient photosynthesis to compensate for both day and night respiration, and in all probability must also allow for some assimilation.

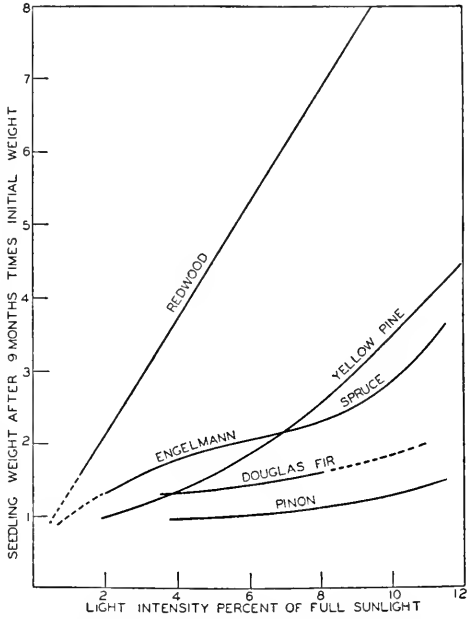

FIG. 89. Relation between light intensity and weights of conifer seedlings after nine months. Data of Bates and Roeser (1928).

Bates and Roeser (1928) studied the effects of low light intensities upon the growth of a number of species of evergreens native to the western United States. The seedlings were exposed to a 200 watt, blue tungsten lamp for 10 hours a day. Differences in light intensity were obtained by growing the seedlings at different distances from the source of illumination. Their results (Fig. 89) show that redwood seedlings were able to maintain their initial dry weight in a light intensity less than 1 per cent of full sunlight, while pinon pine required about 5 per cent, and the other three species were intermediate in their requirements.

Light may exert indirect as well as direct effects upon photosynthesis.

Low light intensities favor stomatal closure and hence may sometimes check photosynthesis by restricting the entrance of carbon dioxide as well as by acting as a direct limiting factor. Similarly high light intensities often cause increased rates of transpiration and hence, indirectly, a reduced water content of the leaf cells, and consequent diminished rates of photosynthesis. A high light intensity also has the usual effect of raising leaf temperatures somewhat above the prevailing temperatures of the surrounding atmosphere and may thus influence photosynthesis indirectly by its effect on thermal conditions within leaves. Very high light intensities also have a destructive effect upon chlorophyll, as has been shown by Ewart (1898) and others.

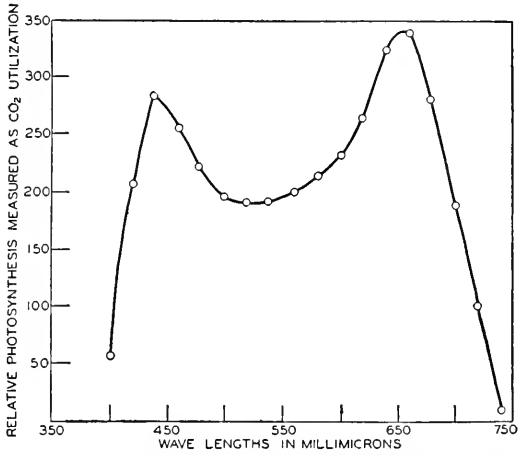

FIG. 90. Relative rates of photosynthesis in different wave lengths of light of equal intensity. Data of Hoover (1937).

3. *Effects of Different Light Qualities upon Photosynthesis.*—Hoover (1937) has recently investigated the effect of different wave lengths of radiation upon the rate of photosynthesis of wheat plants (Fig. 90). By the use of suitable filters he was able to irradiate the plants with narrow spectral bands. All measurements were made with equal intensities of radiation incident upon the plants. The results of his investigation indicate the occurrence of maximum photosynthesis at a wave length of 655 $m\mu$ in the red, and a secondary maximum of 440 $m\mu$ in the blue. The green region of the spectrum is relatively less effective in photosynthesis, presumably because of the smaller proportion of the radiation absorbed by leaves in this range of wave lengths.

There are a number of conditions under which plants growing in their natural habitats are exposed more or less continuously to light of a very dif-

ferent quality from that of full sunlight at the earth's surface. On cloudy days, for example, the intensity of light is not only less than on clear days, but its quality is very different.

Light which has been filtered through the crown of a tree is usually proportionately richer in green rays than direct sunlight because of the greater proportionate absorption in the red and blue portions of the spectrum. This effect upon light quality is most marked in hardwood forests in which the tree crowns form an almost continuous canopy. The herbs, shrubs, and smaller trees growing in such forests are subjected to light which is not only of much lower intensity than full sunlight, but is also different in quality from the light impinging upon the forest canopy.

In habitats of submerged aquatics both the intensity and quality of the light are usually very different from the intensity and quality of the sunlight at the earth's surface. Pure water absorbs radiations in the red-orange portion of the spectrum much more effectively than in the blue-green region. While the absorption coefficients of natural waters for various wave lengths of light vary somewhat, depending upon the substances dissolved or dispersed in the water, in general shorter wave lengths penetrate to greater depths than longer wave lengths. Hence with increasing depth in either fresh or ocean water not only is the intensity of the light reduced, but its quality is greatly modified. Aquatic plants growing at a depth of 20 meters, for example, will be exposed to light proportionately much richer in blue-green rays, although of lower intensity, than those at a depth of 1 meter.

Alpine plants are also exposed to light of different composition than species at lower altitudes. The atmosphere absorbs the shorter wave lengths of the sun's radiation more effectively than the longer ones. Because of the shorter column of atmosphere through which it passes, sunlight at high altitudes is therefore not only more intense than at lower elevations, but is also relatively richer in the shorter wave lengths of visible radiation and the ultraviolet.

4. *Effects of Duration of the Light Period upon Photosynthesis.*—In general a plant will accomplish more photosynthesis in the course of a day if exposed to illumination of favorable intensity for ten or twelve hours than if suitable light conditions prevail for only four or five hours. In arctic regions photosynthesis may occur continuously throughout the 24-hour day. However, even if all external conditions remain favorable the amount of photosynthesis accomplished in a twelve-hour light period will usually not be twice that in a six-hour period, because of certain internal factors (see later) which often become limiting after the process has been in progress for several hours. The length of the daily light period, or *photoperiod,* also

has important effects upon the growth and development of plants which will be discussed in Chap. XXXIII.

The effect of rapid alternations of light and dark in increasing the amount of photosynthesis per unit of light, and the theoretical implications of this effect, have already been discussed in the preceding chapter.

5. *The Induction Period.*—As shown by McAlister (1937), Smith (1937) and others, when a plant is illuminated the rate of photosynthesis is at first low and gradually increases until it reaches a more or less constant value. The length of this "induction period" varies from a minute or two to more than an hour, depending upon the species and environmental conditions. The existence of such an induction period has important theoretical implications which must be considered in any postulated mechanism of photosynthesis.

6. *Solarization.*—Intense illumination inhibits the accumulation of starch in leaves, a phenomenon which is called *solarization.* For example Holman (1930) showed that bean leaves exposed to light of about 6800 foot-candles readily accumulate starch, but that in light of about twice this intensity starch accumulated only when the length of the exposure period was relatively short and then only in relatively small quantities. This effect is believed to involve the process of photosynthesis rather than the secondary reaction of starch synthesis. At the present time there is no entirely satisfactory explanation for the phenomenon of solarization.

Temperature Effects on Photosynthesis.—The determination of the effect of temperature upon photosynthesis in terrestrial plants is complicated by the fact that the leaf temperatures of such plants are seldom the same as atmospheric temperatures. Whenever leaves are exposed to direct illumination, which is almost invariably the situation when photosynthesis is occurring at a rapid rate, their temperatures exceed those of the surrounding atmosphere. It is therefore difficult if not impossible to maintain the temperature of the leaves of land plants at a desired value while they are exposed to light of any considerable intensity. Evaluation of the effect of the temperature of leaves upon photosynthesis is possible only if direct measurement is made of the actual leaf temperature. For this reason many of the more critical studies of the effect of temperature upon photosynthesis have been made with submerged water plants, in which a close thermal equilibrium is maintained between the plant body and the surrounding water.

1. *Temperature Limits of Photosynthesis.*—Photosynthesis can take place over a wide range of temperatures. It has been reported to occur in some species of conifers at temperatures as low as $-35°$ C. and in some kinds of lichens at $-20°$ C. It is said that many temperate zone evergreens are

able to accomplish sufficient photosynthesis in the course of the winter to compensate for winter consumption of carbohydrates in respiration. The occurrence of photosynthesis in the leaves of the cherry laurel (*Prunus laurocerasus*) has been demonstrated at −6° C. Tropical plants cannot carry on photosynthesis at temperatures as low as those at which many temperate zone plants can synthesize simple carbohydrates. In most tropical species photosynthesis apparently will not occur at temperatures below about 5° C. At the other end of the temperature range for photosynthesis stand the species of algae indigenous to hot springs which can survive 75° C. and probably carry on photosynthesis at temperatures close to this value. Many semi-desert and tropical species can withstand air temperatures of 55° C. and probably photosynthesize at temperatures not far below this. In most plants of temperate regions the range of temperatures within which photosynthesis occurs at a relatively rapid rate is about 10-35° C.

2. *The Effect of Temperatures on the Rate of Photosynthesis.*—The general relation between temperature and the rate of photosynthesis is rather complex and can best be discussed in terms of a specific example. If the rate of photosynthesis of an *Elodea canadensis* plant be measured by the bubble-counting method for a number of intervals of time at each of several successively higher temperatures under such conditions that neither light nor carbon dioxide are limiting factors, the results can be plotted as a family of curves such as those shown in Fig. 91. As indicated in this figure the *initial* rate of apparent photosynthesis increases with increase in temperature up to at least 40° C. This initial maximum rate is established soon after the plant is brought to each temperature. The rate of photosynthesis at any given temperature depends, however, not only on the temperature but also on the length of time that the plant has already been at that temperature. There is a marked tendency for the rate of photosynthesis to diminish with time. At 25° C. and even at 30° C. this effect is scarcely perceptible. At 35° C., however, the diminution in photosynthetic rate with time is marked, and at 40° C. it is so pronounced that within a relatively short period, in this experiment, the rate of photosynthesis had declined to nearly a zero value. In other words the higher the temperature, the shorter the period of time for which the maximum rate of photosynthesis can be maintained, and the more rapid the decline in rate after it is once initiated.

The diminution in the rate of photosynthesis with time, particularly marked at higher temperatures, is evidence of the progressively increasing limiting effect of some internal factor, generally called the "time factor." The exact nature of this "time factor" is unknown but a number of suggestions have been made concerning the possible mechanism of this effect:

(1) Accumulation of the end products of the reaction causes a gradual decrease in the rate of photosynthesis. This effect is considered in more detail later in this chapter.

(2) Inactivation of enzymes essential to photosynthesis occurs more rapidly at higher temperatures. The temperature relations of enzymatic reactions

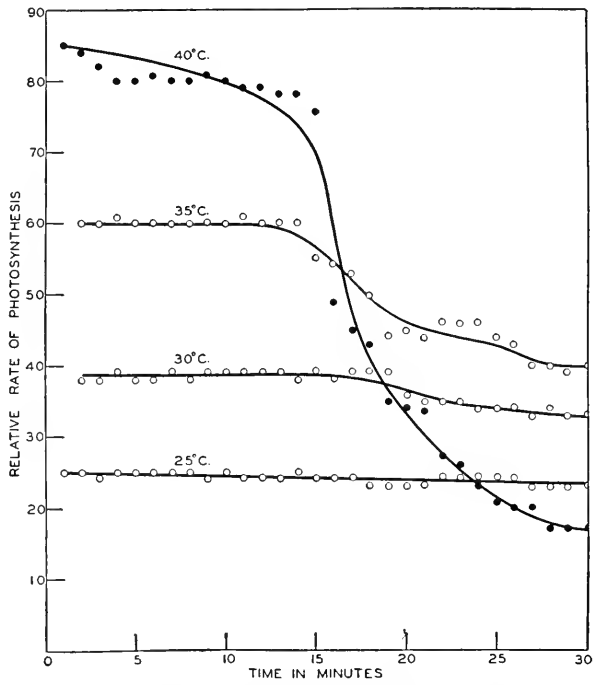

FIG. 91. Relative rates of apparent photosynthesis in *Elodea canadensis* at different temperatures over a period of thirty minutes.

in general are similar to those of the photosynthetic reaction which seems to be evidence in support of this view.

(3) Diffusion of carbon dioxide into the cells of the chlorenchyma may not occur as rapidly as it could be used in photosynthesis, even though the external supply is not limiting.

(4) Respiration increases more rapidly than photosynthesis with increase in temperature, thus resulting in a rapid decrease in the *apparent* photosynthetic rate at high temperatures. That this explanation cannot entirely account for this phenomenon is shown in the subsequent discussion.

(5) High temperatures may have a destructive effect on chlorophyll or possibly on some other protoplasmic factor besides chlorophyll or enzymes.

The first two of the above suggestions appear to be more probable explanations of this phenomenon than the others. It is also quite possible that this effect may result from the composite influence of several factors.

The time factor effect in photosynthesis has also been demonstrated in terrestrial plants. In fact this principle was first pointed out by Blackman and Matthaei (1905) in their classical paper on temperature effects upon the rate of photosynthesis in the cherry laurel and Jerusalem artichoke. In their investigations corrections were made for respiration, indicating that the observed results could not be due to a differential effect of temperature upon photosynthesis and respiration.

The foregoing discussion of the time-temperature relations of photosynthesis indicates that there can be no *optimum* temperature for this process in the usual sense of a certain temperature for each species at which photosynthesis will proceed most rapidly, when no other factors are limiting. The optimum temperature can be defined only with reference to the element of time, and from this standpoint might be considered as the highest temperature at which the initial rate of photosynthesis is maintained for a relatively long period. The optimum temperature for photosynthesis, defined in this sense, varies considerably with different species, and is usually higher in tropical species than in those native to temperate regions.

3. *Influence of Light Intensity upon Temperature Effects on Photosynthesis.*—Temperature effects upon photosynthesis are further complicated by the fact that they may differ according to the light intensity to which the plants are exposed. As pointed out in the last chapter the temperature coefficient of photosynthesis at relatively low light intensities approximates one, while at higher light intensities it approximates two. At relatively low light intensities, therefore, raising the temperature, at least within the range of about 15-30° C. (Warburg, 1919), has little effect on the rate of photosynthesis, because the rate of the process is limited by its photochemical phase. At relatively high light intensities, however, the rate of photosynthesis is approximately doubled for each 10° C. rise in temperature, subject to the limitations imposed by the time factor effect. A logical conclusion which may be drawn from this finding is that temperature has very little effect on the rate of photosynthesis in plants growing in deep shade or in plants exposed to the low light intensity of cloudy or foggy days.

The Rôle of Water in Photosynthesis.—Less than 1 per cent of the water absorbed by a plant is used in photosynthesis. It therefore seems probable that the indirect effects of the water factor upon photosynthesis are more

pronounced than its direct effects. In other words deficiency of water as a raw material is not commonly a limiting factor in photosynthesis. Nevertheless, a reduction in the water content of leaves generally results in a decrease in the rate of photosynthesis (Fig. 92). There are probably two principal ways in which this effect can be exerted: (1) reduction in the diffusive capacity of the stomates resulting from the decreased leaf water content, and (2) reduction in the hydration of the chloroplasts and other parts of the protoplasm.

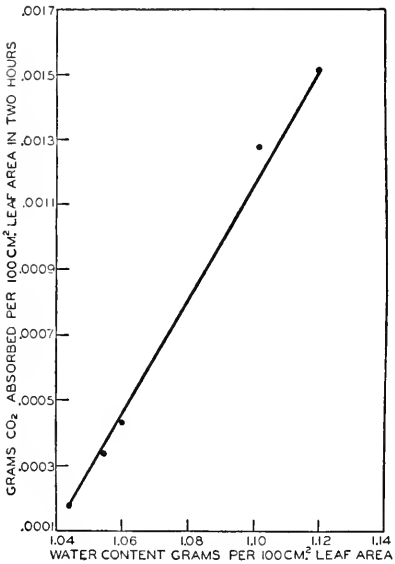

FIG. 92. Relation between rate of photosynthesis in attached leaves of *Abutilon darwinii* and their water content. Data of Dastur (1925).

There is no question that decrease in the water content of leaves causes a reduction in the diffusive capacity of the stomates. The results of Mitchell (1936) indicate, however, that reduction in the diffusive capacity of the stomates does not have as marked an effect upon photosynthesis as is sometimes supposed. Leaves of tomato and *Pelargonium* were found to utilize carbon dioxide approximately as rapidly under high humidities as under low ones, although under the latter condition the stomates appeared to be almost entirely closed. The probable explanation of this seemingly anomalous finding is that stomates which appear closed to microscopic observation are not entirely closed to the passage of gases. It should also be recalled that the diffusive capacity of only slightly open stomates is much greater than the area of the apertures would suggest (Chap. XIII).

In general it appears that the rate of photosynthesis is less affected by reduction in leaf water content than the rate of transpiration. This is indicated by the results of Heinicke and Childers (1936) who determined the average rates of photosynthesis and transpiration over a week's period in two apple trees, one of which was well watered while the other was growing in soil which was gradually drying out. The rate of photosynthesis of the plant in the dry soil was about half as great as in the plant which was watered. The rate of transpiration, however, was only about one-fourth as great in the former plant as in the latter.

The evidence in favor of the view that the decreased hydration of the protoplasm which accompanies a reduction in cell turgidity causes a decreased rate of photosynthesis comes chiefly from experiments with water plants. Walter (1929), for example, has studied the effect upon their rate of photosynthesis of immersing plants of *Elodea canadensis* in sucrose solutions of various concentrations. The greater the concentration of the sucrose solution the less the turgidity of the cells, the less the hydration of protoplasm and the slower the rate of photosynthesis. In one experiment the rate of photosynthesis was appreciably retarded by immersion of the plants in an 0.3 weight molar solution and almost entirely stopped in an 0.7 weight molar solution of sucrose with an osmotic pressure of about 18 atmos. Plasmolysis of the cells occurred at a solution concentration of between 0.3 and 0.4 weight molar. The rate of respiration, on the other hand, was practically unaffected by sucrose solutions at any concentration up to and including 1.0 weight molar.

Since *Elodea canadensis* is a submerged aquatic with thin leaves which bear no stomates, any reduction in the rate of photosynthesis resulting from a diminution in cell turgor is most likely due to direct effects upon the hydration of the protoplasm of the photosynthesizing cells. It is probable, however, that a greater reduction in protoplasmic hydration is required in most land plants than in such submerged species before the rate of photosynthesis is appreciably retarded.

Other External Factors Influencing Photosynthesis.—1. *Oxygen.*—The results of a number of investigators suggest very strongly that oxygen is necessary for the process of photosynthesis. The amount required, however, apparently is very small as a reduction in the partial pressure of the oxygen to one-hundredth of that present in the atmosphere has been found by Willstätter and Stoll (1918) to have no effect upon the rate of photosynthesis in a number of species. The apparent necessity of oxygen for photosynthesis suggests that the respiratory mechanism of the cells is probably in some way involved in the process. Oxygen is probably never a limiting factor for photosynthesis in plants growing under natural conditions.

2. *Mineral Elements.*—Briggs (1922) has shown that the rate of photosynthesis in bean plants deficient in potassium, magnesium, iron, or phosphorus is less than in similar plants provided with all of the necessary mineral elements. The mechanisms of such effects are necessarily very complex and are incompletely understood, although one way in which deficiency of mineral salts may influence photosynthesis adversely is by checking development of chlorophyll.

3. *Various Chemical Substances.*—The effects of a number of miscellaneous compounds upon photosynthesis have been studied by various investi-

gators. Some of the results obtained are of considerable theoretical importance, but a detailed discussion of these phenomena is out of place in an introductory textbook. As one example we may mention the effects of ether and chloroform. Either of these substances, even in very small concentrations, will arrest photosynthesis. Respiration is much less sensitive to the effects of these two anaesthetics than photosynthesis, so by exposure of a plant to chloroform or ether in the proper concentration it is possible to completely inhibit photosynthesis without greatly influencing the rate of respiration.

A point of considerable theoretical interest is the fact that narcotics inhibit only the light reaction, while cyanides inhibit only the dark reaction of photosynthesis.

The Effect of Internal Factors upon the Rate of Photosynthesis.—1. *Chlorophyll Content.*—In their extensive study of the relationship between chlorophyll content and photosynthesis Willstätter and Stoll (1918) devised the *photosynthetic number* ("Assimilationszahl") as an index to this relation. The photosynthetic number is the number of grams of carbon dioxide absorbed per hour per gram of chlorophyll. These two investigators have studied the relation between chlorophyll content and photosynthesis in green-leaved and yellow-leaved varieties of the same species (Table 34). In this experiment the leaves were exposed to strong light in an atmosphere of 5 per cent carbon dioxide at 25° C.

TABLE 34—THE RELATION BETWEEN PHOTOSYNTHESIS AND CHLOROPHYLL CONTENT IN GREEN AND YELLOW-LEAVED VARIETIES OF ELM AND ELDER (DATA OF WILLSTÄTTER AND STOLL, 1918)

Species	Variety	Chlorophyll content of 10 g. fresh leaves in mg.	CO_2 absorbed per hr. in mg.		Photosynthetic number
			Per 10 g. leaf tissue	Per dm.^{2} leaf surface	
Elm	Green	16.2	111	21	6.9
Elm	Yellow	1.2	98	24	82.
European Elder	Green	22.2	146	34	6.6
European Elder	Yellow	0.81	97	21	120.

As shown in this table the rate of photosynthesis in green-leaved varieties is not much in excess of that in yellow-leaved varieties of the same species, and when expressed in terms of the photosynthetic number the yellow-leaved varieties are much more efficient per unit of chlorophyll present. Other investigations by the same workers also seem to point to the conclusion that there is no proportional relationship between chlorophyll content and photo-

synthesis in the leaves of the higher species of plants. In other words, it appears that chlorophyll content of the leaves is seldom the limiting factor in photosynthesis in such species even when all external conditions are favorable for the process.

Results somewhat at variance with the above have been obtained by Emerson (1929) and Fleischer (1935) working with the unicellular alga *Chlorella.* The latter investigator controlled the chlorophyll content in different cultures of this alga by limiting the quantity of iron, magnesium, or nitrogen present. When the chlorophyll content was controlled by restricting the amount of iron available, a direct proportional relation was found to exist between the chlorophyll content and the rate of photosynthesis if no other factors were limiting. A similar relation was found to exist when the chlorophyll content was limited by regulating the amount of nitrogen available but lack of magnesium was found to check the rate of photosynthesis before it checked the rate of chlorophyll formation. This suggests that magnesium plays another role in photosynthesis in addition to its influence on chlorophyll synthesis.

2. *Hydration of the Protoplasm.*—That the hydration of the protoplasm is an important internal factor influencing photosynthesis has already been shown in the discussion of the water factor in photosynthesis.

3. *Leaf Anatomy.*—The rate of photosynthesis in any leaf will be partly conditioned by the anatomy of that leaf. The size and distribution of the intercellular spaces, the relative proportions and distribution of palisade and spongy layers, the size, position, and structure of the stomates, the thickness of the cuticular and epidermal layers, the amount and position of sclerenchyma, proportion and distribution of non-green mesophyll tissues, the size, distribution and efficiency of the vascular system, etc. will all influence the rate of photosynthesis. The effects of the structure of leaves upon the rate of photosynthesis result principally from influences upon the rate of entrance of carbon dioxide, upon the intensity of light penetrating to chlorenchyma cells, upon the maintenance of the turgidity of the leaf cells, and upon the rate of translocation of soluble carbohydrates out of the photosynthesizing cells.

4. *Protoplasmic Factors.*—Evidence from various types of experiments, some of which have been presented on the foregoing pages, indicates conclusively that certain other conditions resident in the protoplasm of plant cells, other than chlorophyll content and hydration, influence the rate of photosynthesis. It is not possible to say with certainty just how many such protoplasmic factors there are, but indications regarding two of them are fairly specific. The apparent necessity of oxygen for photosynthesis suggests very strongly that the respiratory mechanism of the cells is in some way involved in the process. Secondly, various lines of evidence suggest that enzymatic

systems are indispensable for the occurrence of photosynthesis, although no specific enzyme or enzymes has ever been identified with the process.

5. *Accumulation of the End Products of Photosynthesis.*—During rapid photosynthesis the carbohydrates produced in the process or in immediately following secondary reactions accumulate in the photosynthesizing cells more rapidly than they are translocated towards other tissues. Accumulation of carbohydrates in chlorenchyma cells exerts a retarding effect upon photosynthesis (Kurssanow, 1933). In excised leaves of the grape, for example, photosynthesis ceases completely when the percentage of carbohydrates present becomes equivalent to 17 to 25 per cent of the dry weight of the leaf (Saposchnikoff, 1891). The mechanism of this effect is not positively known, but it seems probable that two principal causes are involved. Accumulation of sugars in the cell sap may result in an increase in its diffusion pressure deficit and hence in a decrease in the hydration of the protoplasm. Reduction in the hydration of the protoplasm, as we have already seen, results in a diminished rate of photosynthesis. Furthermore accumulation of large quantities of starch within the chloroplasts may interfere mechanically with their efficient operation in photosynthesis.

Daily Variations in the Rate of Photosynthesis.—Thomas and Hill (1937) measured the daily variation in rates of apparent photosynthesis for

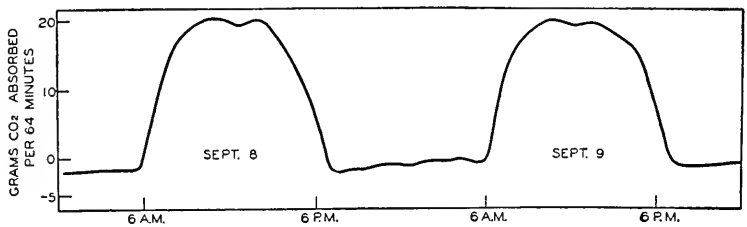

FIG. 93. Daily variations in the rate of apparent photosynthesis of alfalfa. Data of Thomas and Hill (1937).

small plots of alfalfa and wheat (Fig. 93). Well watered plots of alfalfa six feet square were enclosed in transparent celluloid cabinets and air circulated through each cabinet at rates ranging up to several hundred cubic feet per minute. The consumption of carbon dioxide by the plants was determined by measuring the difference in the concentration of this gas in the inflowing and outflowing streams of air. No measurement was made of the quantity of carbon dioxide liberated as a result of soil respiration in the enclosed plots, and this source of error must be considered in evaluating the results. In

general, as with the apple tree previously described, the rate of photosynthesis under these conditions showed a close correlation with the light intensity.

When the daily cycle of photosynthesis is measured in small plants, *i.e.* under such conditions that light is a limiting factor for little or none of the leaf area, the resulting curves often have a different shape from that shown in Fig. 93, even on clear days. Often the peak of the curve occurs during the mid-morning hours (Kostychev *et al.,* 1926, and others); sometimes the rate reaches a maximum during the forenoon, decreases to a minimum during mid-day, and rises to a secondary maximum later in the afternoon, before falling again to zero (Kurssanow, 1933). The explanation for daily photosynthetic cycles of the types just described is not clear but they are probably due to a temporary limiting effect of certain internal factors, such as accumulation of the products of photosynthesis, or to temporary mid-day closure of the stomates (Chap. XIII).

Discussion Questions

1. What environmental factors would be of first and second importance as limiting factors in photosynthesis outdoors in the east central United States in July? in January? the year round? In a greenhouse in the same region in July? in January? In the semi-desert regions of the southwest, considered the year round?
2. If in the morning a given rate of photosynthesis were found to occur in a plant under favorable conditions of light, CO_2 concentration, temperature, and soil water supply, would you expect to find the same rate in the afternoon if all environmental factors remained unchanged? Explain.
3. If half the leaves were removed from a plant how will the daily rate of photosynthesis per unit of leaf area of the remaining leaves compare with the rate if the leaves had not been removed? Explain. Would the distribution of the removed leaves on the plant make any difference?
4. Explain in some detail why, other conditions being favorable, a plant growing under conditions of a favorable soil water supply will accomplish more photosynthesis in the course of a day than one growing where the soil water supply is inadequate.
5. Would increasing the intensity of the sunlight falling on a corn plant by the use of mirrors at noon on a cloudless day result in any appreciable increase in the rate of photosynthesis? Explain.
6. Under what conditions would the addition of water to the soil around a growing plant be expected to result in an appreciable increase in the rate of photosynthesis? no very great change? a measurable decrease?
7. How would you expect the rate of photosynthesis to behave in plants subjected to continuous illumination?
8. If the CO_2 concentration of the atmosphere is usually the limiting factor during the summer months for plants well exposed to light, why is it possible to increase the production of many crop plants by adding fertilizers to the soil?
9. Why does adding carbon dioxide to the atmosphere of greenhouses in the northeastern United States during the winter months often have little or no beneficial effect on plants?

10. Why should a deficiency of water have a smaller retarding effect on photosynthesis than on transpiration?
11. Absorption spectra of the chlorophylls (Fig. 81) indicate much less absorption of light in the green portion of the spectrum than in the blue and red, yet photosynthesis is not greatly less in the green than in the red and blue (Fig. 90). How can these facts be reconciled?
12. Assuming "standard day" conditions would you expect a greater rate of photosynthesis in a plant in perfectly quiet air or in a similar plant subjected to a moderate breeze?
13. Assuming otherwise "standard day" conditions would you expect the photosynthesis of an entire apple tree to be greater on a perfectly clear day or one with scattered cumulus clouds in the sky? Explain.
14. If increasing the intensity of light to which a leaf is exposed results in an increase in the rate of photosynthesis does this indicate conclusively that light was initially a limiting factor?
15. *Elodea* plants immersed in dilute solutions of sodium bicarbonate show an immediate increase in rate of photosynthesis if the temperature is raised from 30° to 35° C. The rate of photosynthesis in leaves of many land plants exposed to full sunlight increases only slightly or not at all with the same rise in temperature. Explain.

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CHAPTER XXII

CARBOHYDRATE METABOLISM

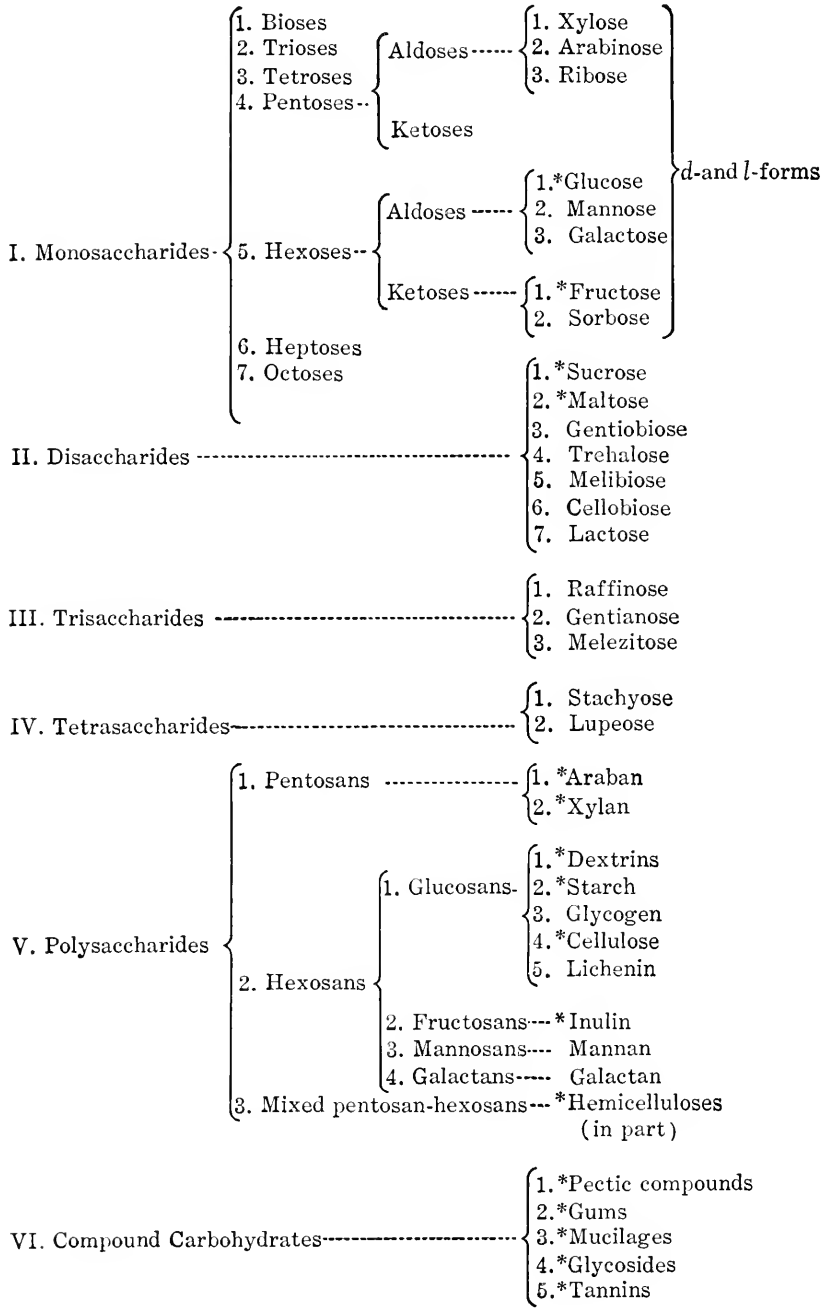
The simple sugars synthesized in photosynthesis belong chemically to the group of compounds known as the carbohydrates. As the name indicates these compounds contain the elements carbon, hydrogen, and oxygen, the latter two usually, but not invariably, being present in the same ratio in which they are found in the water molecule, *i.e.* two hydrogen atoms for every oxygen atom. Generally speaking the term carbohydrate refers to aldehydic or ketonic derivatives of alcohols of the aliphatic series, and their condensation products. The significance of this statement will become clearer in the light of the following discussion.

A large number of different kinds of carbohydrates have been isolated from plants, and doubtless many more remain to be discovered. They compose the bulk of the dry matter of plants. Although some of the carbohydrates are of universal occurrence in plants, or practically so, others seem to be restricted to a very few species. Certain carbohydrates are the important structural components of the cell walls of plants, others are probably integral parts of the protoplasm, some are in solution in the cell sap, while large quantities of others accumulate in plant cells as insoluble storage products.

Classification of the Carbohydrates.—A classification of the carbohydrates is presented in Table 35. This table includes all of the more important types of carbohydrates, but only those known to be of considerable significance in plant metabolism are listed by specific name in the right hand column of the table. Those which are known to be of general and fairly abundant occurrence in green plants have been further designated by prefixing their names with a star.

The monosaccharides are the group of carbohydrates from which no simpler carbohydrates can be produced by hydrolysis. They are classified according to the number of carbon atoms which they contain. Although monosaccharides of all of the seven groups included in Table 35 have been identified, only the 5-carbon atom (pentose) and 6-carbon atom (hexose) monosaccharides are important in plants. Most of the sub-groups of monosaccharides can be further divided into aldoses and ketoses. Aldoses are monosaccharides containing an aldehydic group ($-\overset{H}{\overset{|}{C}}=O$), while ketoses contain a ketonic group ($=C=O$).

TABLE 35 - A CLASSIFICATION OF THE CARBOHYDRATES



The disaccharides are formed by the condensation of two molecules of monosaccharides. All of the important disaccharides are formed by condensation of two hexose molecules. The combining monosaccharide molecules may both be the same kind or of different kinds. Trisaccharides are formed by the condensation of three molecules of monosaccharides, and tetrasaccharides by the condensation of four monosaccharide molecules. Upon hydrolysis these types of carbohydrates produce two, three, or four monosaccharide molecules respectively. The important tri- and tetrasaccharides are all formed by the condensation of molecules of hexose sugars.

The polysaccharides are produced by the condensation of a large number of monosaccharide molecules. In most of the polysaccharides all of the condensing molecules are of the same kind, although some are formed by the condensation of molecules of different kinds of monosaccharides.

The compound carbohydrates are formed by the condensation of one or more monosaccharide molecules with non-carbohydrate molecules. Some of the groups listed under this heading, notably the tannins, can be considered as only remotely related to the carbohydrates.

General Properties of the Sugars.—The mono-, di-, tri-, and tetrasaccharides are collectively called the sugars. All of these compounds possess the property of sweetness and all of them are white, more or less crystalline compounds which are soluble in water.

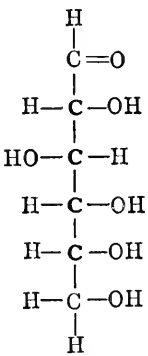
1. *Reducing and Non-reducing Sugars.*—All of the monosaccharides and some of the more complex sugars act as reducing agents. This action is due to the presence of an aldehydic or ketonic group in the sugar molecule. Those compound sugars in which the linking of the monosaccharides has occurred in such a manner that the aldehydic or ketonic groups have lost their usual reactivity are non-reducing. Sugars are commonly classified on this basis as *reducing sugars* or *non-reducing sugars*. The reducing action of sugars is most commonly determined by means of Fehling's or Benedict's solution, in which, upon heating, a reducing sugar converts cupric hydroxide into cuprous oxide. The latter compound separates from the solution in the form of a reddish precipitate. These solutions are used not only for the qualitative demonstration of reducing sugars but for their quantitative estimation, since the quantity of precipitate formed, although not directly proportional, bears a definite relation to the amount of sugar taking part in the reaction.

2. *Optical Activity.*—Most of the soluble carbohydrates are, like many other organic compounds, optically active when in solution. The *optical activity* of a solution of carbohydrate refers to its property of rotating the plane of polarized light. The *specific rotatory power* of a sugar is expressed in terms of the number of degrees of angular rotation of the plane of polarized

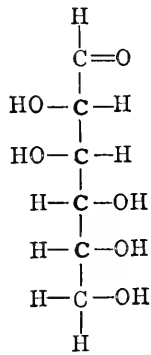
light caused by a solution made in the proportion of 1 g. of the sugar to 1 cc. of solution, observed through a depth of 10 cm. at a temperature of 20° C. Measurements of the rotatory power of substances are made with an instrument known as a *polarimeter*. Compounds which rotate the plane of polarized light to the right (in a clockwise direction) are called *dextrorotatory;* those which rotate it to the left (in a counter-clockwise direction), *levorotatory*.

3. *Isomerism.*—An important fact regarding the sugars is that many isomers may exist with the same molecular formula. There are two important types of these, ordinary chemical isomers, and *stereoisomers*. The former are due to differences in atomic groupings within the molecule. The difference between two isomers of this type may be seen by comparing the structural formulas for *d*-glucose and that for *d*-fructose (see later). Although the molecular formula of both is the same ($C_6H_{12}O_6$) *d*-glucose is an aldehyde derivative while *d*-fructose is a ketone derivative.

Stereoisomers are illustrated diagrammatically for *d*-glucose and *d*-mannose. Here exactly the same atomic groupings are present, but they are arranged in different patterns around the asymmetric carbon atoms.



d-glucose

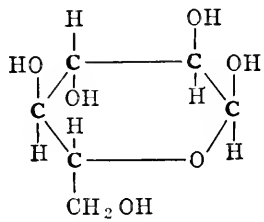


d-mannose

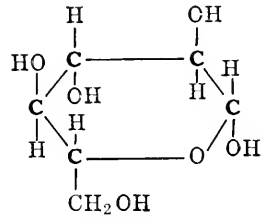
Two stereoisomeric forms exist for each of the simple sugars, the arrangement for *l*-glucose and *l*-mannose being the exact reverse of the above patterns. One important difference between these two forms of any compound is that one (usually the *d* or *dextro* form), rotates the plane of polarized light to the right, while the other form (usually the *l* or *levo* form), rotates it an equal number of degrees to the left. Mixtures of equimolar quantities of these two forms are optically inactive. In sugars, however, the prefixes *d* and *l* do not necessarily refer to the direction of their specific rotatory power as they do when prefixed to the names of all other organic compounds. *d*-fructose, for example, is strongly levorotatory. Sugars are designated *d* or *l,* not because

of the direction of their rotatory power, but because of their structural relationships to *d* and *l*-glucose respectively.

4. *Ring Structure.*—Although it has long been customary to write the formulas of monosaccharides as straight carbon chains, in recent years it has become evident that the structure of such sugars is best represented by a ring type of formula (Haworth, 1929). The 1-5 ring form of *d*-glucose exists in α and β forms depending upon the position of attachment of the H and OH groups to the aldehydic carbon atom, which has now also become asymmetric:



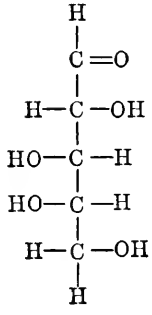
α *d* glucose



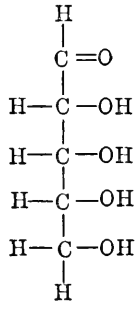
β *d* glucose

The α and β ring forms of glucose are stable compounds, but other ring forms of these compounds, known as *gamma* sugars, also exist.. These are unstable, highly reactive compounds and apparently have only a transitory existence, but because of their lability probably play an important role in metabolic processes.

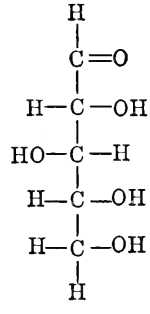
The Monosaccharides.—The *pentoses* are five carbon atom sugars ($C_5H_{10}O_5$). Eight isomeric aldoses and four isomeric ketoses having this formula are known to be possible, but only three of these—*d*-xylose, *l*-arabinose, and *d*-ribose are definitely known to occur in plants. Although the molecules of the pentose sugars apparently also have a ring structure, only the more familiar straight-chain formulas are given here for these three sugars:



d-arabinose



d-ribose



d-xylose

The few analyses of plant tissues which have been made for the pentoses indicate that they never represent more than 1 per cent of the dry weight of plant tissues. Arabinose and xylose form pentosans upon condensation (see later) while ribose is a hydrolytic product of nucleic acid (Chap. XXVI).

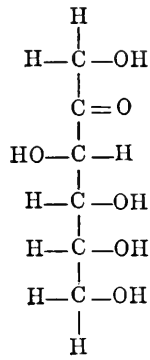
Pentoses are also hydrolytic products of the gums, mucilages, pectic compounds, and hemicelluloses. They are therefore important building blocks in the synthesis of certain more complex plant carbohydrates. The origin of the pentoses in plants is unknown. It is possible that they are synthesized directly in photosynthesis; an alternative possibility is that they are formed from the hexose sugars.

The *hexoses* are six carbon atom sugars represented by the molecular formula $C_6H_{12}O_6$. Sixteen stereoisomeric aldoses and eight stereoisomeric ketoses having this formula are known to be possible. Of all these, only two, *d*-glucose (an aldose) and *d*-fructose (a ketose), are commonly found in plants in the free state. *d*-mannose and *d*-galactose, both aldoses, and *d*-sorbose, a ketose, are hydrolytic products of a number of the more complex carbohydrates.

d-glucose (also called dextrose, blood sugar, corn sugar, or grape sugar) is the most familiar of all the hexoses, and is of widespread occurrence in plants. It is apparently present in practically every living plant cell. *d*-glucose is dextrorotatory (hence the name "dextrose"); its specific rotatory power at 20° C. being + 52.7°. Apparently transformation of glucose to fructose, as well as the reverse reaction, occurs readily in plant cells. Glucose is frequently assumed to be the first product of photosynthesis, and while there is little direct evidence that this is true, there can be little doubt that it is *one* of the first products. Glucose is probably the sugar most frequently oxidized in respiration, and is probably also a common translocation form of carbohydrate. Its condensation products include starch, dextrins, cellulose, and glycogen. It is also a hydrolytic product of certain di-, tri-, and tetrasaccharides.

d-mannose and *d*-galactose are both found in plants in the free state only in traces, and are evidently only transitory products in the metabolism of plants. Their condensation products are mannosans and galactosans respectively, both discussed later. Galactose is a fundamental constituent of the pectic compounds and is also one of the sugars produced upon the hydrolysis of lactose, raffinose, stachyose, gums and mucilages.

d-fructose (also called levulose, or fruit sugar), like glucose, is nearly always present in the cells of the higher plants. The straight-chain structural formula for *d*-fructose is as follows:

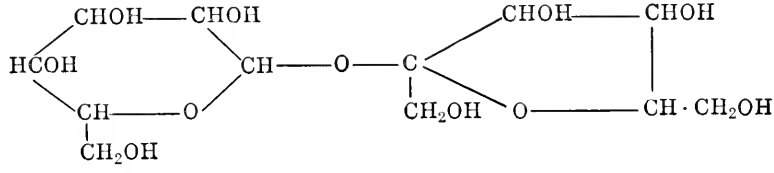


Fructose is levorotatory (hence the name "levulose"), its specific rotatory power at 20° C. being $-92.5°$. It is especially abundant in many fruits in which it often exceeds the amount of either glucose or sucrose present. Fructose may be one of the simple sugars produced directly in the process of photosynthesis. Fructose can probably be oxidized in respiration, and undoubtedly is readily translocated from cell to cell in plants. Condensation of fructose molecules produces *inulin*, an important storage carbohydrate in some species of plants. Fructose is also one of the hydrolytic products of sucrose and of several of the tri- and tetrasaccharides.

The Disaccharides.—The three most important disaccharides found in plants are sucrose ("cane sugar"), maltose and cellobiose.

Sucrose is produced by the condensation of equimolar quantities of glucose and fructose (Chap. XX). Under certain conditions digestion of sucrose to glucose and fructose occurs under the influence of the enzyme sucrase (Chap. XXVII). Most plant tissues contain larger quantities of sucrose than any of the other sugars. In sugar cane stalks from 12 to 20 per cent of the fresh weight is sucrose; in the roots of the sugar beet, it represents from 15 to 20 per cent of the fresh weight. Considerable quantities of sucrose are also found in the sap of the sugar maple and in certain of the sorghums.

The probable structural formula of sucrose is as follows:



The right hand portion of the sucrose molecule may be regarded as de-

rived from a gamma fructose molecule, the left hand portion from an *d*-glucose molecule.

Sucrose is dextrorotatory, its specific rotatory power being + 66.5°. When hydrolyzed by acids or the enzyme sucrase the resulting mixture, the so-called "invert sugar", is levorotatory. Although glucose and fructose are present in the resulting mixture in equimolar quantities, the latter is more strongly levorotatory than the former is dextrorotatory.

Maltose is widely distributed in plants, but is seldom present in more than small amounts. It is produced by the condensation of two molecules of glucose, apparently under the influence of the enzyme maltase, as follows:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{Maltase}} \underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O$$

Maltase also accomplishes the reverse reaction (digestion) of maltose. This sugar is an intermediate product in the synthesis and digestion of starch as described later. Maltose is a reducing sugar and is strongly dextrorotatory (specific rotatory power + 137°).

Cellobiose is a reducing dissacharide which is produced from cellulose by the action of the enzyme cellulase. Upon hydrolysis it yields two molecules of glucose. Cellobiose differs from maltose in that it is formed by the condensation of two molecules of β *d*-glucose, while a molecule of maltose results from the condensation of two molecules of α *d*-glucose.

Other disaccharides found in plants are listed in Table 35.

Tri- and Tetrasaccharides.—Trisaccharides are sugars with the molecular formula $C_{18}H_{32}O_{16}$. *Raffinose* is a non-reducing trisaccharide present in small quantities in many of the higher plants and in fungi. It is found in appreciable quantities in cotton seeds and sugar beets. Upon complete hydrolysis it yields one molecule each of galactose, glucose, and fructose. Partial hydrolysis may yield either fructose or melibiose, or galactose and sucrose depending upon the catalyst used.

The trisaccharide *gentianose* has been found in the roots of the yellow gentian (*Gentiana lutea*). Upon partial hydrolysis it yields one molecule each of fructose and gentiobiose; upon complete hydrolysis one molecule of fructose and two of glucose. *Melezitose* is a very sweet trisaccharide which has been found in the sap of several conifers (European larch, Douglas fir, Scrub pine). The drops of sweet sap ("honey dew") exuded by such species after attacks of sucking insects are often rich in this carbohydrate. Upon complete hydrolysis this sugar yields three molecules of glucose.

Tetrasaccharides are represented by the molecular formula $C_{24}H_{42}O_{21}$. An example is *stachyose* which has been isolated from the roots of the hedge

nettle (*Stachys tubifera*). Upon complete hydrolysis this carbohydrate yields one molecule of glucose, one of fructose and two of galactose.

The Polysaccharides.—The polysaccharides are carbohydrates formed by the condensation of large numbers of monosaccharide molecules. Those occurring in the plants fall largely into the three groups *hexosans, pentosans* and mixed *pentosan-hexosans*. The latter group corresponds roughly to the so-called "hemicelluloses." The polysaccharides are not sweet like the sugars, and they are not soluble in water. All of them, however, are more or less hydrophilic, and hence are frequently found as components of hydrophilic colloidal systems. In this property the pentosans, in general, greatly surpass the hexosans.

1. *The Pentosans.*—These compounds have the empirical formula $(C_5H_8O_4)_n$ and are the condensation products of arabinose and xylose, the corresponding pentosans being the *arabans* and *xylans*. The molecules of these compounds apparently consist of long chains of arabinose or xylose residues, similar in structure to cellulose and starch molecules described later. Arabans and xylans apparently occur principally in the cell walls of plants. In some species, such as the cacti, they are also important constituents of the mucilaginous materials present in the cells, and contribute largely to the hydrophilic properties of such substances. Arabans are one of the constituents of cherry, peach, and plum gum, while xylans are found very commonly in wood, straw, corncobs, and seed coats. Xylans may constitute as much as 15 per cent of the woody tissues of some trees.

2. *The Hexosans.*—The important polysaccharides which are synthesized in plants from *d*-glucose are cellulose, starch, dextrins, and glycogen. All of these, as well as the hexosans formed from other hexoses, are represented by the empirical formula $(C_6H_{10}O_5)_n$.

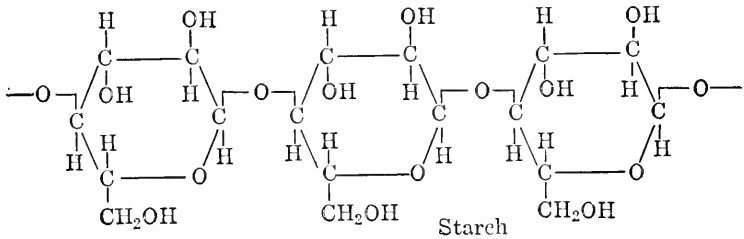
Cellulose is the principal constituent of the cell walls of all of the higher plants (Chap. VI). In terms of absolute amounts it is probably the most abundant organic compound present on the earth. Much of the cellulose as it occurs in the plant is in intimate mixture with, or encrusted by, other materials. Some fibers, however, are practically pure cellulose. Fibers of the cotton plant, for example, are composed of about 91 per cent cellulose, 8 per cent water, and only 1 per cent of other substances.

Cellulose "molecules" are long, ribbon-like structures. These chain-like molecules are built up by the linear condensation of β *d*-glucose molecules. Each cellulose molecule therefore consists of a chain of at least 100 and probably many more glucose residues (Chap. VI) linked together by oxygen bridges.

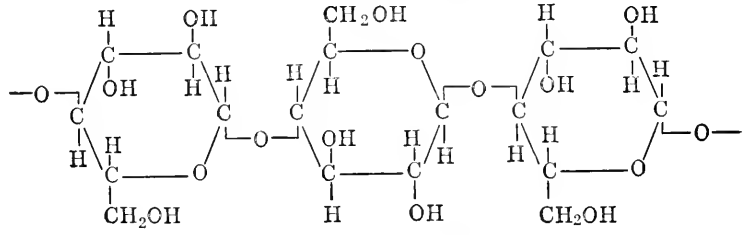
Chemically cellulose is relatively inert, being insoluble in water and all

organic solvents. One of the few liquids which will dissolve it is an ammoniacal solution of copper hydroxide (Schweitzer's reagent). Concentrated sulfuric acid will gradually hydrolyze cellulose into glucose, while dilute sulfuric acid causes it to swell and converts it into "hydrocellulose." In sodium hydroxide solutions of about 15 per cent, cellulose swells, also producing a "hydrocellulose"; this effect is the basis of the process of mercerization. Stepwise hydrolysis of cellulose results first in the production of *cellobiose,* a disaccharide, each molecule of which is in turn hydrolyzed into two molecules of glucose.

Next to cellulose, *starch* is undoubtedly the most abundant hexosan occurring in plants. Starch "molecules" are built up by the linear condensation of many molecules of α *d*-glucose, each glucose residue being connected to the next by an oxygen bridge. The difference between starch and cellulose is shown in the following two structural formulas, one of which represents three of the glucose residues in a starch molecule, the other three of the glucose residues in a cellulose molecule.



Starch



Cellulose

Starch is an almost universal form of storage carbohydrate, since there are very few of the higher green plants in which this compound does not accumulate in some organ of the plant. Of the relatively small group of plants which do not form starch, the onion, leek, snowdrop, and dahlia are some of the more familiar species. The failure of the plants in this group to synthesize starch is sometimes ascribed to a lack of the proper enzymes, although in some species it is apparently due to failure of a high enough

concentration of sugars to develop to permit the occurrence of starch synthesis.

A temporary accumulation of starch occurs in the leaves of most species during photosynthesis (Chap. XX). Starch synthesis also takes place in the cells of many other tissues. Starch accumulates in the xylem rays, xylem parenchyma, phloem parenchyma, cortex and pith of the stems of many species, as well as in tubers, bulbs, corms, underground rhizomes, fruits and seeds.

Starch is insoluble in water, but upon boiling is converted into a colloidal sol. Under suitable conditions it may also form gels. Upon hydrolysis with acids, or digestion with "diastase" (a mixture of the enzymes amylase and maltase) starch is converted, first into dextrins, then into maltose, and finally into glucose. It is generally considered that there are several dextrins, which are produced step-wise in the hydrolysis of starch as follows:

Starch → Amylodextrin → Erythrodextrin → Achroodextrin → Maltose → Glucose

Amylodextrin, erythrodextrin, and achroodextrin are differentiated by the colors which they give with I_2KI solution, these being blue, reddish brown,

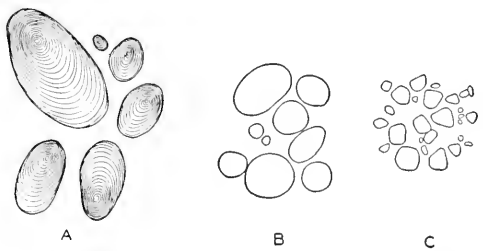


FIG. 94. Starch grains. (*A*) from potato tuber, (*B*) from wheat grain, (*C*) from corn (maize) grain.

and colorless respectively. The presence of starch itself is usually demonstrated by the purplish-blue color which it gives with this same reagent.

With very few exceptions starch is present in the cells of plants in the form of definite grains or granules. These grains vary greatly in size, as well as in the number which may be present in a single cell. The shape of starch grains, however, is so nearly constant for a given species that the origin of the starch in starch-containing products such as flour can often be determined by examining a small sample of the product under the microscope (Fig. 94).

Starch grains are usually built up of a number of layers or lamellae

which have been deposited around a central locus. In "simple" starch grains there is only one locus of deposition; in the "compound" type there are a number of such loci.

As far as is known, starch grains are formed only in chloroplasts and leucoplasts. The grains formed in the chloroplasts are usually small, and seem to be produced within the interior of the chloroplast. Most of the larger and more prominent starch grains produced in plant cells are synthesized by the leucoplasts. Apparently each leucoplast produces only a single starch grain. It is not certain whether the starch grain in its growth by the accretion of new layers ruptures the outer membrane of the leucoplast, or whether the outermost layer of the leucoplast stretches as the starch grain enlarges and thus remains as a thin membrane around the grain.

Starch grains are hydrophilic, usually containing about 13-15 per cent of water. The material present cannot be converted quantitatively into glucose, indicating that some other substances are present in the grain. The amounts of these other compounds are small, however, seldom equalling even 1 per cent of the total dry matter present. They include such compounds as fats, proteins, tannins, phosphates, and other mineral compounds.

The *dextrins* are a group of compounds formed as intermediate products in the hydrolysis of starch, and undoubtedly in its synthesis also. The dextrins do not often accumulate in plants, usually being present only as transitory products. They form colloidal sols in water. Although their constitution is represented by the same formula as that for starch, the number of unit $C_6H_{10}O_5$ groups composing a dextrin molecule is smaller than in starch.

Glycogen (sometimes called "animal starch") is a hexosan which serves as a storage carbohydrate in animal tissues, being especially abundant in the muscles and liver. It is unknown in green plants, but found in many of the fungi, being especially abundant in yeasts.

The only well-known levulosan is *inulin* which is accumulated and serves as a storage product in a number of plants, especially members of the composite family. Some species in which inulin is found are the dahlia, chickory, salsify, dandelion, Jerusalem artichoke, and goldenrod. Inulin is seldom if ever found in the aerial organs of plants, but may constitute as much as 15 per cent of the dry weight of some underground parts. Some species (Jerusalem artichoke) accumulate starch in the aerial parts, but inulin in the underground portions. Inulin is a white powder-like compound which forms colloidal sols in water. It is dispersed in the cell sap of those cells in which it accumulates and can be precipitated as crystals in the cells by immersing them in alcohol. Inulin is hydrolyzed in plants to fructose by the enzyme *inulase*.

3. *"Hemicelluloses."*—This term has no critical chemical significance, but is used to designate a heterogeneous group of polysaccharides which are of widespread occurrence as cell wall constituents in plants. Superficially most hemicelluloses bear some resemblance to cellulose, but differ from it in being easily hydrolyzed by dilute acids and alkalies.

The hemicelluloses include the mixed pentosan-hexosans as classified in Table 35. The term is frequently loosely employed to include also various pentosans and hexosans such as xylans, galactans and mannans. Apparently all of these types of compounds may be present as intimate mixtures in the cell walls of plants.

Hemicelluloses are widely distributed in plants. They are found especially in seed coats, endosperms, nutshells, stony fruits, and in woody tissues. The endosperms of the seeds of nasturtium, date, and onion, for example, contain hemicelluloses. The hemicelluloses present in many seeds are used as food by the young seedlings during germination. Compounds of this type, although cell wall constituents, may therefore serve as reserve foods. The hemicelluloses found in the cell walls of the woody tissues of some trees, as for example the apple tree, also serve as reserve food which is digested and utilized when growth of the stems is resumed in the spring.

Compound Carbohydrates.—These are compounds which have been derived from carbohydrates and non-carbohydrate groups, the latter, however, often being close chemical relatives of the carbohydrates.

Among the substances usually classed in this group are the *pectic compounds.* These are important constituents of the cell walls of plants. Three types of pectic compounds are generally recognized: *pectic acid, pectin*, and *protopectin.* All of the pectic compounds are markedly hydrophilic and form sols and gels with water.

Recent investigations indicate that pectic acid has a structure analogous to that of cellulose (Bonner, 1936). Pectic acid "molecules" seem basically to be built up by the condensation of long chains of galactose molecules. Many of the galactose residues have, however, been converted into galacturonic acid residues and some have been modified to arabinose residues. The products of hydrolysis of pectic acid are galacturonic acid, galactose, and arabinose, but they are not present in constant proportions. In other words the so-called pectic acid is not a compound in the strict chemical sense of the word, but a group of similar compounds. Pectic acid does not occur in plants in the free state, but its salts, especially calcium pectate, are of widespread occurrence. This salt is the most important constituent of the middle lamella and acts as a cementing material between the adjacent primary cell walls.

Pectin apparently differs from pectic acid principally in the presence of

methyl groups in the molecule. All or a portion of the carboxyl groups may be methylated.

Household fruit jellies owe their "gelling" property to the pectin derived from the fruits. Neither protopectin nor pectic acid can be used in making such jellies. Because of its commercial exploitation as an aid in jelly-making pectin is the best known of the pectic compounds. The use of commercial pectin is of the greatest value in preparing jellies from fruit products which do not themselves contain sufficient pectin to gel readily. Pectin gels for household purposes require the presence of three components: pectin (usually from 0.2 to 0.7 per cent), sugar (usually 65 to 70 per cent), and sufficient acid to maintain a pH of about 3.0 to 3.2.

Protopectin apparently differs from pectin in the greater length of the molecular chain. Protopectin occurs most abundantly in the primary cell walls, in which it is present in the intermicellar material.

The cell walls of many fleshy fruits, such as apples, are especially rich in protopectin. During storage of apples and other fruits protopectin is gradually converted into "soluble" pectin (Carré and Horne, 1927). As fruits become overripe further degradation of the pectin to galacturonic acid, galactose, and arabinose may occur. Because of dissolution of the pectic compounds of the middle lamella the cells of overripe fruits gradually separate from each other and the fruit becomes soft and flabby.

As already noted in Chap. XVII root hairs also seem to be coated with pectic substances, but their exact chemical nature is unknown.

The *gums* are complex compound carbohydrates that are somewhat similar in composition to the pectic compounds. Upon hydrolysis they yield hexoses, or pentoses, or both, and a complex organic acid. Gum arabic is an example of such compounds. It forms as an exudate from the branches of some of the tropical species of acacia. Upon hydrolysis gum arabic produces arabic acid, galactose, and arabinose. Gum tragacanth is exuded from the stems of species of the genus *Astragalus* and is similar in its properties to gum arabic. The gummy exudates formed on the stems of cherry, plum, and peach trees also belong to this group of substances. Some of the gums readily form colloidal sols in water, as for example gum arabic, others such as cherry gum merely swell in water.

The *mucilages* are complex compound carbohydrates which form slimy colloidal systems with water. Mucilages are found in the cells of cacti and other succulents, are exuded by the epidermal hairs of many plants, coat the surface of the seeds of some species (flax), and are abundant on the outside walls of many aquatic species. Agar-agar and other similar products of the marine algae are usually classed as mucilages. Knowledge of the chemistry

of the mucilages is almost totally lacking; present indications are that they are fundamentally similar to the gums.

The *glycosides* are compounds formed by the reaction between a sugar (most commonly glucose) and one or more compounds which are non-sugars. All glycosides may exist in two forms, α or β, but most of them which occur in plants are of the β type. Although of widespread occurrence in plants the glycosides are never present in large quantities. They may be found in almost any part of the plant. In a pure state they are mostly levorotatory, crystalline, colorless, bitter, and soluble in either water or alcohol. All β-glycosides can be hydrolyzed by the enzyme *emulsin* (Chap. XXVII) or by dilute mineral acids. Several hundred different glycosides have been isolated from plant tissues. Their role in the metabolism of plants, if any, is obscure although it is possible that they may serve in a minor way as storage foods.

Several representative glycosides will be discussed briefly in order to indicate more clearly the general nature of these compounds.

Salicin is found in the bark and leaves of the willow tree. Upon hydrolysis with emulsin it yields glucose and the alcohol *saligenol* according to the following equation:

$$\underset{\text{Salicin}}{C_{13}H_{18}O_7} + H_2O \xrightarrow{\text{Emulsin}} C_6H_4\begin{cases}CH_2OH\\OH\end{cases}_{\text{Saligenol}} + \underset{\text{Glucose}}{C_6H_{12}O_6}$$

Amygdalin has probably been studied more than any other glycoside. It occurs in the seeds of apples, peaches, and plums, as well as in the leaves of cherries and peaches. Upon hydrolysis with emulsin it produces glucose, hydrocyanic acid, and benzaldehyde:

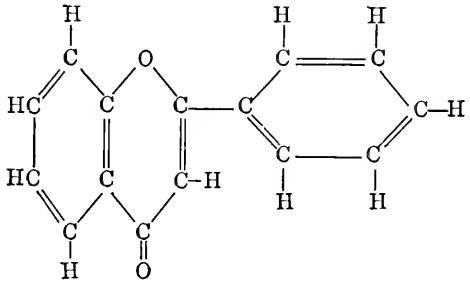
$$\underset{\text{Amygdalin}}{C_{20}H_{27}NO_{11}} + 2\,H_2O \xrightarrow{\text{Emulsin}} 2\,\underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\substack{\text{Hydrocyanic}\\\text{acid}}}{HCN} + \underset{\text{Benzaldehyde}}{C_6H_5CHO}$$

Domestic animals are sometimes poisoned from eating plant material such as cherry leaves which contain this glycoside. The toxic action of amygdalin is due to the liberation of hydrocyanic acid upon hydrolysis. The enzyme (emulsin) and the glycoside apparently are kept apart in the cells since usually rapid hydrolysis of amygdalin does not occur unless the cells are crushed in some way.

Sinigrin is called the mustard oil glycoside. It is found in the black mustard (*Brassica nigra*), and is hydrolyzed as follows:

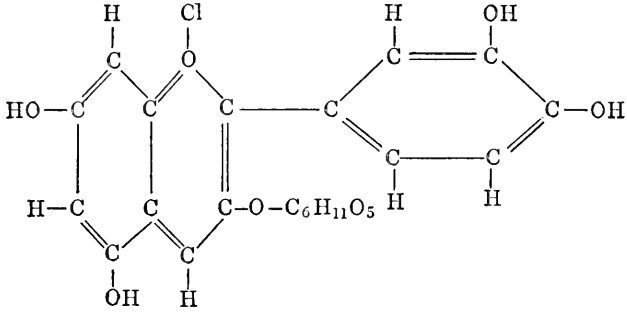
$$\underset{\text{Sinigrin}}{C_{10}H_{16}O_9NS_2K} + H_2O \xrightarrow{\text{Emulsin}} \underset{\substack{\text{Allyl isothio-}\\\text{cyanate (“Mus-}\\\text{tard oil”)}}}{C_3H_5CNS} + \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\substack{\text{Potassium}\\\text{hydrogen}\\\text{sulfate}}}{KHSO_4}$$

The Anthocyanins.—Most of the red, blue, and purple pigments of plants belong to the group known as the *anthocyanins*. These compounds are glycosides which have been formed by condensation of a sugar (usually glucose) with one of the compounds belonging to a group known as the *anthocyanidins*. The anthocyanidins are derivatives of the compound *flavone* which has the following structural formula:



Most of the anthocyanidins are derived from flavone by reduction of the oxygen atom of the middle ring, substitution of one or more hydroxyl groups and addition of a chlorine atom by the reduced oxygen.

The chemical formula of *chrysanthemin chloride*, a representative anthocyanin which occurs in species of the genus *Chrysanthemum*, illustrates the molecular structure of these compounds:



A number of chemically different anthocyanins have been isolated from seed plants in which they are of widespread occurrence. They are also found in some species of the lower plant groups. The anthocyanins are water soluble, and when present in the cells are usually dissolved in the cell sap, the cytoplasmic membranes being impermeable to them. Less commonly these pigments are found in plant cells in the form of crystals or amorphous solid bodies. Practically all anthocyanins are red in acid solutions and many

of them change in color through violet to blue as the H-ion concentration of the medium decreases. Red pigmentation due to anthocyanins is frequently found in flowers, fruits, bud scales, developing leaves, and less commonly in stems, mature leaves (red cabbage, copper beech, red coleus, etc.) and other plant parts. The reds and purplish reds developed in autumn foliage are also due to anthocyanins. Blue and purple pigmentation due to the presence of anthocyanins is restricted largely to flowers and fruits.

Factors Influencing the Synthesis of Anthocyanins in Plants.—Although anthocyanin pigments have been extensively studied in the laboratory from the chemical standpoint, very little is known of their actual synthesis in plants. Certain factors have been recognized, however, which are usually correlated with anthocyanin production in plants.

1. *Genetic Constitution.*—The potentiality of producing anthocyanins is controlled by hereditary factors. The red maple, for example, produces anthocyanins in its flowers, young stems, and developing leaves, while the leaves turn a brilliant red just before leaf fall in the autumn. On the other hand, the sugar maple, a closely related species, produces anthocyanins less abundantly, while the black maple, a variety of the sugar maple, only rarely produces anthocyanins in readily observable quantities. Similarly different varieties of the same species may differ in the hereditary potentialities for the production of anthocyanins in the flower parts. Extensive studies of the genetics of anthocyanin flower color production have been made in some species.

2. *Accumulation of Soluble Carbohydrates.*—One metabolic condition which seems to be universally correlated with anthocyanin formation is the presence of a relatively high concentration of simple sugars in the cells. The presence of large amounts of these substances does not necessarily predispose the tissues to anthocyanin formation, but this apparently is a necessary prerequisite. The frequently observed anthocyanin formation in the developing leaves and shoots of many species is correlated with their high sugar content at that time. An injury of stems or leaves which interferes with the translocation of foods from those organs often favors anthocyanin production in such parts. Such injuries may be mechanical in origin, or may be due to insect injuries or fungous infections. Similarly the leaves of many species contain more sugar in the autumn at the time they turn red than earlier in the season.

3. *Temperature.*—Lowering of the temperature often favors anthocyanin formation. It seems probable that the effect of this factor on the synthesis of anthocyanins is largely indirect. Reduction in temperature nearly to the freezing point is known to favor the transformation of insoluble carbohydrates into soluble forms as described later. Reduced temperatures also

result in a diminution in the respiration rate, which favors sugar accumulation. The accumulation of the soluble carbohydrates in the cells in turn favors anthocyanin formation.

4. *Light.*—The effects of light upon anthocyanin synthesis may also be partly indirect, due to the influence of this factor upon photosynthesis. Light also has a direct effect upon the formation of anthocyanins in some plant organs. Autumnal red coloration, for example, usually develops only in leaves which are directly exposed to the light. If one leaf is covered by another during the period of anthocyanin synthesis in the autumn the lower leaf will "photograph" the upper clearly as an area devoid of red pigment.

Anthocyanin formation is favored principally by the shorter visible and the ultraviolet rays. Arthur (1932, 1936) has found that it is possible to color apples picked late in the summer artificially under light sources supplying the shorter wave lengths of the visible spectrum or ultraviolet. The most effective portion of the spectrum was found to be the range from 290-313 $m\mu$, *i.e.* in the ultraviolet, although all wave lengths up to 600 $m\mu$ resulted in considerable coloring. Areas of the skin of the apples covered with opaque paper before being exposed to these light treatments failed to develop any red pigment.

While in some plant organs light appears to be necessary for anthocyanin formation, and in others probably favors synthesis of these pigments, there are some plants in which anthocyanins are synthesized in the absence of light. The roots of the beet furnish a familiar illustration of this phenomenon. Similarly the young leaves of both beet and radish will synthesize anthocyanins, even if the seeds are germinated in the absence of light.

5. *Available Nitrogen.*—Reduction in the available nitrogen supply in the soil favors anthocyanin formation. Under such conditions a smaller proportion of the carbohydrates in the plant is utilized in the synthesis of amino acids (Chap. XXVI); the consequent relatively high concentration of carbohydrates favors anthocyanin formation. Anthocyanin formation is a common symptom of nitrogen deficiency in many species of plants.

6. *Drought.*—Drought conditions in general favor anthocyanin formation. Deficiency of water, in many species at least, favors the conversion of insoluble carbohydrates into soluble carbohydrates. Furthermore a deficiency of soil water tends to reduce the absorption of nitrates, which also favors the accumulation of carbohydrates. The formation of red pigments during droughts is therefore principally due to indirect effects of soil water deficiency upon the metabolism of the plant.

7. *Oxygen.*—Oxygen is apparently necessary for anthocyanin synthesis, and seems to be chemically combined in this process. This is seldom if ever

a limiting factor in the natural formation of anthocyanin pigments. Contrariwise the destruction of anthocyanins in plant tissues is accompanied by the release of considerable quantities of oxygen.

The Anthoxanthins.—Exposure of the petals of almost any white flower to ammonia vapor will cause them to turn yellow. This is due to the presence in such tissues of what may be regarded as a colorless form of one of the *anthoxanthins*. Like the anthocyanins these compounds are chemically related to flavone and usually occur in plants in the form of glycosides. Most of the anthoxanthins are colorless or nearly so as they occur in the plant but upon extraction and treatment in various ways their typical yellow or orange color develops. Like the anthocyanins they are water-soluble, and are usually found in the cell sap. In some plant tissues, the anthoxanthins present are yellow in color. The yellow pigment in the inner bark of the black oak (*Quercus velutina*) is due to an anthoxanthin called *quercitrin*. Similar pigments occur in the wood of various other species (osage orange, sumac, etc.), and in certain fruits (oranges). Some flowers, as for example yellow snapdragons, owe their yellow color to anthoxanthins. The color of most yellow flowers is due, however, to the plastid pigments carotene and the xanthophylls.

Autumnal Leaf Coloration.—The most spectacular display of pigmentation in the plants of temperate regions is the annual autumnal coloration of leaves, especially of woody species. The "turning" of leaves in the autumn is not due, as is commonly believed, to the effects of frost. In fact early frosts will greatly reduce the abundance and brilliance of the autumn leaf colors by killing or severely injuring the leaves before the pigments reach their maximum development. The sequence of events leading to the coloration of leaves in the autumn seems to be about as follows: In late summer or early autumn chlorophyll synthesis in the leaves ceases, while the destruction of the chlorophyll already present apparently proceeds at an accelerated rate. As the chlorophyll disappears the residual yellow plastid pigments—carotene and xanthophylls—become apparent. The yellow color of the leaves of many species at this season, as for example, the tulip polar, sycamore, and birch, is due to the disappearance of the chlorophyll which has masked the presence of the yellow pigments during the summer season. The golden yellow effect produced in some leaves, such as those of beeches, is due to the presence in the cells of a brownish pigment, probably a tannin, in addition to the yellow pigment.

The more prominent colors in most autumn landscapes, however, are the various shades of red and purplish red which develop in the leaves of such species as the red maple, many oaks, sumac, dogwood and black gum. These

are due to the synthesis of anthocyanins in the leaf cells of these species. Autumnal development of anthocyanins is favored by periods of bright, clear, dry weather, during which cool, but not freezing temperatures prevail. From the preceding discussion of the factors favoring anthocyanin formation it is evident that such meteorological conditions should be almost ideal for the synthesis of these compounds.

Tannins.—These are a rather heterogeneous group of complex compounds of common occurrence in plants. Upon hydrolysis they produce certain complex acids, nearly always a sugar (usually glucose) and sometimes other substances. The proportion of sugar found in the hydrolytic products of the tannins is relatively small, however. While the tannins may be regarded as remotely related to the glycosides, their properties are distinctive as compared with the glycoside type of compound, and they must be considered as a separate class of substances.

Tannins vary greatly in amount from one species to another. They are sometimes present in the cell sap but are of more frequent occurrence in the cell walls, often accumulating in very considerable amounts in dead tissues. Tannins are found in the leaves of many species such as tea (15 per cent of the dry weight) oaks, and many conifers. The woody tissues of many species contain tannins. The bark of oaks, chestnut, hemlock, sumac and other species is very rich in tannins. In some species of oak they may compose as much as 40 per cent of the dry weight of the bark. Unripe fruits of some species (persimmon, plum, etc.) contain relatively large quantities of tannins.

Carbohydrate Transformations in Plants.—Of the many carbohydrate transformations which occur in plant cells, the conversions of glucose to starch, and of starch back to glucose probably are of most frequent occurrence. Certain conditions within plant cells are known to favor the condensation of glucose to starch; others the digestion of starch to sugar:

1. *Temperature.*—Low temperatures in general favor the starch to sugar transformation in plant cells. This fact is clearly illustrated by the seasonal behavior of the carbohydrates in the leaves of evergreen species and in woody stems as already discussed in Chap. XI.

Another interesting example of a shift in the starch-sugar equilibrium with temperature occurs in potato tubers (Hopkins, 1924). If stored at too low a temperature a gradual accumulation of sugars will occur in the tubers at the expense of the starch present. This accounts for the sweet taste which is sometimes found in potatoes purchased on the market. Contrary to popular opinion this sweetening is not necessarily due to a freezing of the tubers, since it has been found that temperature of the inception of sugar

accumulation is about 5 or 6° C. (41-43° F.). Storage of potatoes at temperatures in this range just above the freezing point will therefore induce sugar formation in the tubers.

Apparently it is only in a zone of intermediate temperatures that starch formation at the expense of sugars is favored in potato tubers, as relatively high temperatures (35-45° C.) also appear to favor hydrolysis of starch to sugar. It is possible, however, that this shift in equilibrium towards sugars at the higher temperatures is a result of the reduction in water content which may be induced by such temperatures (Wolff, 1926).

Similar effects of temperature on carbohydrate equilibria are shown by other species. In the sweet potato sucrose accumulates rapidly at the expense of starch at temperatures below a critical range of 13-16° C. but above this range most of the stored carbohydrate remains in the form of starch (Hopkins and Phillips, 1937). In ripening banana fruits hydrolysis of starch occurs rapidly at temperatures between 21 and 26° C. but there is practically no starch hydrolysis at 10° C. It is evident that the critical temperature ranges for hydrolysis and synthesis of starch vary greatly according to species.

2. *Water Content.*—In wilting leaves much of the starch present is digested to sugars (Molisch, 1921; Ahrns, 1924). A relatively high water content apparently favors "starchiness" in the leaf tissues of many species, while a severe reduction in water content induces a conversion of starch into sugar. Sucrose accumulates as well as simple sugars during wilting. That many species of plants do accumulate sugars rather than starch under drought conditions has already been pointed out in Chap. XVIII. In certain succulent plants, such as cacti, desiccation favors the accumulation of polysaccharides, rather than soluble sugars (Spoehr, 1919), so evidently no single general principle regarding the influence of the water content of the cells upon carbohydrate equilibria within them can be formulated.

3. *H-ion Concentration.*—The action of enzymes is very sensitive to the H-ion concentration of the medium wherein they operate (Chap. XXVII). Since most of the carbohydrate transformations occurring in plants are probably catalyzed by enzymes, the pH of the medium in which these reactions occur may have an important effect upon the rate of these transformations. Apparently the pH of the medium may influence not only the rate of an enzymatic reaction but may also influence its direction. The reversible carbohydrate transformations occurring in the guard cells of the stomates afford the best known example of this effect.

4. *Concentration of Sugars.*—Theoretically, a high concentration of sugars in a cell would favor starch synthesis, and *vice versa.* The daytime accumulation of starch in green leaves which are photosynthesizing rapidly

is apparently due to the maintenance of a relatively high concentration of sugars in the cell sap, since under conditions unfavorable for rapid photosynthesis the accumulation of starch is greatly reduced. During the hours of darkness, when a high sugar concentration of the cells is no longer maintained by photosynthesis, the accumulated starch is rapidly reduced in amount by transformation to sugars, in which form it is translocated out of the leaf.

Discussion Questions

1. Outline diagrammatically the stages in the synthesis of the principal polysaccharides from the hexose produced in photosynthesis.
2. Why does sweet corn lose its sweetness soon after being picked?
3. Wild cherry leaves are much more poisonous to cattle after they have been cut for a few days than when they are fresh. Explain.
4. What will be the effect of girdling a stem on the time of appearance and intensity of red color in leaves of species in which anthocyanins are synthesized?
5. Heavy fertilization of red coleus plants greatly decreases the intensity of the red color of the leaves. Explain.
6. Why are leaves of some species such as the black cherry usually yellow at the time of falling if they drop from the tree during a midsummer drought, but generally red at the usual time of abscission in the autumn?
7. Which of the common plant pigments are water soluble? which are fat soluble? Give reasons for your answers.

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CHAPTER XXIII

FAT METABOLISM

Fats and fat-like substances are present in every living cell. They are produced as a result of certain chains of reactions which can occur in most and probably all actively metabolizing plant cells. Fats and their chemical relatives serve in a number of indispensable roles in plants as the succeeding discussion will show.

Esters.—Fats and most other lipids (see below) are compounds of the type which are termed *esters*. The formation of a typical ester is represented by the following equation:

$$\underset{\text{Ethyl alcohol}}{C_2H_5OH} + \underset{\text{Acetic acid}}{CH_3COOH} \rightleftarrows \underset{\text{Ethyl acetate}}{CH_3COOC_2H_5} + H_2O$$

As indicated in this equation the reaction between ethyl alcohol and acetic acid results in the production of the ester, ethyl acetate, and water. All esters may be regarded as resulting from similar reactions between an alcohol and an acid. Fats and most other lipids are esters of fatty acids of relatively high molecular weight with complex alcohols.

The Lipids.—The fats and certain other compounds, more or less closely related chemically, are often called the *lipids*. In general lipids may be considered to be compounds which are insoluble in water, but soluble in fat solvents (ether, chloroform, benzene, etc.) and which structurally are either esters of fatty acids, or hydrolytic products of such esters. The following classification (Bloor, 1925) is a useful one:

I. Simple Lipids—esters of fatty acids with various alcohols.
   1. Fats—esters of fatty acids with glycerol, *i.e. glycerides*.
   2. Waxes—esters of fatty acids with alcohols other than glycerol.

II. Compound Lipids—esters of fatty acids containing groups in addition to alcohol and fatty acid radicals.
   1. Phospholipids (often called phosphatides)—substituted fats containing phosphoric acid and nitrogen. *Lecithin* is the best known.
   2. Glycolipids—compounds of fatty acids with a carbohydrate, also containing nitrogen. Compounds of this group are not definitely known to occur in plants.

III. Derived Lipids—certain substances derived from compounds in the above groups by hydrolysis.
   1. Fatty acids of various series.
   2. Sterols—mostly alcohols of large molecular weight, soluble in fat solvents. Examples: *cholesterol* ($C_{27}H_{45}OH$), and *ergosterol* ($C_{28}H_{43}OH$). Collectively the sterols found in plants are often called *phytosterols.*

Fatty Acids.—There are two principal groups of fatty acids, the saturated and the unsaturated. With very few exceptions the fatty acids found in living organisms contain an even number of carbon atoms. The general formula of fatty acids of the saturated series is $C_nH_{2n+1}COOH$. The following are the principal acids in this series:

Formic	$HCOOH$	or	CH_2O_2
Acetic	CH_3COOH	or	$C_2H_4O_2$
Propionic	$CH_3(CH_2)COOH$	or	$C_3H_6O_2$
Butyric	$CH_3(CH_2)_2COOH$	or	$C_4H_8O_2$
Caproic	$CH_3(CH_2)_4COOH$	or	$C_6H_{12}O_2$
Caprylic	$CH_3(CH_2)_6COOH$	or	$C_8H_{16}O_2$
Capric	$CH_3(CH_2)_8COOH$	or	$C_{10}H_{20}O_2$
Lauric	$CH_3(CH_2)_{10}COOH$	or	$C_{12}H_{24}O_2$
Myristic	$CH_3(CH_2)_{12}COOH$	or	$C_{14}H_{28}O_2$
Palmitic	$CH_3(CH_2)_{14}COOH$	or	$C_{16}H_{32}O_2$
Stearic	$CH_3(CH_2)_{16}COOH$	or	$C_{18}H_{36}O_2$
Arachidic	$CH_3(CH_2)_{18}COOH$	or	$C_{20}H_{40}O_2$

The first four homologues in the above series do not commonly occur in fats. Caproic, caprylic, and capric acids occur as glycerides in palm and coconut oils. Lauric acid has been found as a glyceride in laurel, coconut, and palm oils. Palmitic acid is found as glycerides in bayberry wax, palm oil, and many other plant and animal fats. Stearic acid is similarly a constituent of many plants and animal fats. Arachidic acid is found abundantly as a glyceride in peanut oil.

The molecules of the unsaturated fatty acids contain one or more pairs of carbon atoms united by a double bond. Molecules of such acids are not entirely "saturated" with hydrogen as they possess the capacity of combining with two additional atoms of hydrogen for each double bond present. The best known of the unsaturated fatty acids is oleic acid which contains one double bond located at the midpoint of the carbon chain. Its formula can therefore be indicated as follows: $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$. Oleic acid is the most abundant of all unsaturated plant fatty acids.

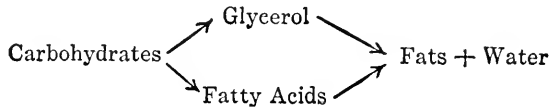
Linoleic acid ($C_{18}H_{32}O_2$) is an example of a fatty acid which contains two double bonds. This acid is an important constituent of cottonseed oil. Linolenic acid ($C_{18}H_{30}O_2$), found in linseed oil, contains three double bonds. A number of other less common unsaturated fatty acids have been isolated from the tissues of plants and animals.

All of the unsaturated fatty acids combine with hydrogen, oxygen, or the halogens. The "drying" properties of linseed, sunflower, and certain other oils are due to the combination of the highly unsaturated fatty acid radicals of the oil with oxygen of the air resulting in the formation of solid, waxy compounds.

None of the fat-forming fatty acids—saturated or unsaturated—is appreciably soluble in water. The lower members of the saturated series are liquids at ordinary temperatures, while those containing ten or more carbon atoms are solids. Most of the unsaturated fatty acids found in plants are liquids at ordinary temperatures. In general the fatty acids resemble the fats proper in most of their properties.

Fat Synthesis.—It is generally considered that fats, being insoluble in water, cannot readily diffuse from cell to cell, although the actual evidence for this supposition is not very substantial. It seems probable, therefore, that fats are usually synthesized in the cells in which they occur. Fats contain the same three elements—carbon, hydrogen, and oxygen—as the carbohydrates, but the proportion of oxygen to carbon is less in fats than in the carbohydrates. In other words the carbon in fats is in a more highly reduced form than in the carbohydrates. When in a liquid state fats are often called *oils*. Regardless of the physical state of these compounds, however, all are included under the chemical term of fats.

Fats are synthesized in living organisms by the condensation of one molecule of the trihydric alcohol glycerol (glycerine) with three molecules of the same or different fatty acids. Both the glycerol and the fatty acid molecules are synthesized from carbohydrates. The general scheme of fat synthesis may be represented as follows:



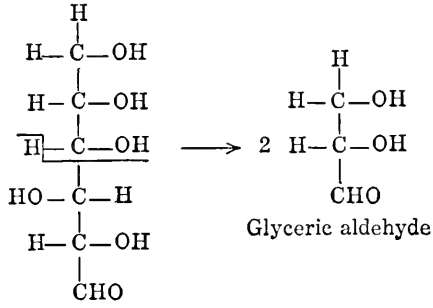
During the maturation of many oily seeds an increase in oil content occurs concurrently with a decrease in the quantity of carbohydrate present (Table 36). This indicates that the carbohydrates in the seed are being converted into fats, and is in accord with the generally accepted theory of fat synthesis.

TABLE 36—CHANGES IN THE PROPORTIONS OF FATS AND CARBOHYDRATES IN THE KERNEL OF ALMOND SEEDS DURING MATURATION (DATA OF LE CLERC DU SABLON, 1896)

Date of collection	Per cent fat	Per cent glucose	Per cent sucrose	Per cent starch and dextrins
June 9	2	6	6.7	21.6
July 4	10	4.2	4.9	14.1
August 1	37	0	2.8	6.2
September 1	44	0	2.6	5.4
October 4	46	0	2.5	5.3

The preceding discussion indicates that there are three important steps in the synthesis of fats in plants: (1) synthesis of glycerol, (2) synthesis of fatty acids, and (3) condensation of fatty acids and glycerol resulting in the formation of fats.

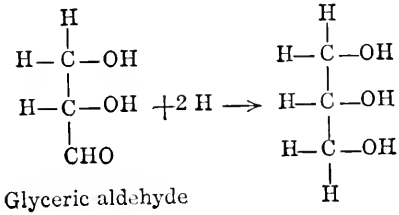
1. *Synthesis of Glycerol.*—Glycerol is a heavy, colorless, viscous liquid which is miscible with water in all proportions. Although the chemical reactions by which glycerol is produced from carbohydrates in plant cells are not known, it is not difficult to postulate how this process *might* take place. Cleavage of a glucose molecule at the center of the carbon chain will give rise to two molecules of glyceric aldehyde, thus:



Glucose

Glyceric aldehyde

Reduction of the glyceric aldehyde would result in the production of glycerol:

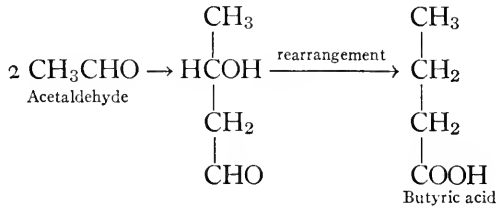


Glyceric aldehyde

Glycerol

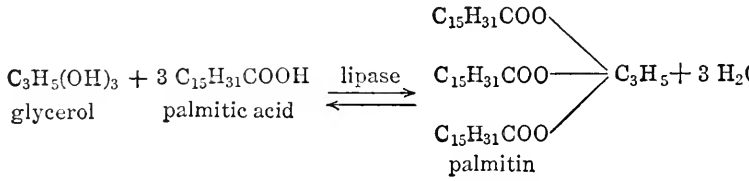
Reduction reactions in the cells of living organisms are not brought about by the action of free hydrogen, but reducing systems whereby hydrogen is transferred from one kind of molecule to another are known to occur in plant cells (Chap. XXX). Synthesis of glycerol in plant cells may therefore occur in essentially the manner which has just been described. The production of glycerol from the hexose sugar involves reduction of the carbon atoms which requires energy. The energy with which this process is accomplished comes from the process of respiration.

2. *Fatty Acid Synthesis.*—Practically nothing is actually known of the manner whereby fatty acids are synthesized from carbohydrates in plant cells, although several theories of the possible mechanism of this process have been advanced. It is generally considered that the fatty acids do not arise directly from the carbohydrates, but from some simpler compound, such as acetaldehyde (CH_3CHO), which is produced by decomposition of the carbohydrates. By the combination of molecules of acetaldehyde, a fatty acid with a longer chain of carbon atoms could be built up, as follows:



By similar reactions fatty acids of still longer carbon chains could be constructed. There is little positive evidence, however, in favor of this view of fatty acid synthesis, except the fact that acetaldehyde is commonly found in plant cells. The conversion of carbohydrates to fatty acids, by whatever series of reactions this is accomplished, involves reduction of the carbon atoms and hence requires energy. The energy utilized in fatty acid synthesis, as in the synthesis of glycerol, is furnished by the process of respiration.

3. *Condensation of Fatty Acids and Glycerol.*—There is little doubt that this is the final stage in the process of fat synthesis. Using palmitic acid for purposes of illustration the equation for this reaction can be written as follows:



The condensation of fatty acids and glycerol in plant cells is supposed to be

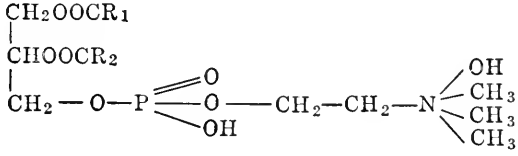
catalyzed by the enzyme lipase. In the fat palmitin, represented in the above equation, the three fatty acid radicals are all of the same species. As mentioned previously, however, the three fatty acid radicals in a fat may be all different, or two may be alike and the third different. Naturally occurring fats are usually mixtures of a number of chemically different kinds of fats of which palmitin is simply one common example.

Evidence that this is the final step in the synthesis of fats may be regarded as conclusive. This is indicated on the one hand by the fact that when fats are hydrolyzed in the laboratory by the action of acids, alkalis, or extracts of the enzyme lipase the products of the reaction are fatty acids and glycerol, suggesting that these are also the compounds from which the fats are synthesized. Digestion of fats to fatty acids and glycerol in plant cells is also accomplished by lipase (Chap. XXVII).

Furthermore synthesis of fats from fatty acids and glycerol under the influence of lipase can actually be demonstrated in the laboratory. If glycerol, a fatty acid, and an extract of lipase be mixed in the proper proportions, precautions being taken to keep the mixture sterile, and incubated for a suitable period of time, the disappearance of fatty acids from the mixture can be demonstrated. A part of the fatty acids present is removed from the mixture under such conditions and tied up in the formation of fats.

The synthesis of fats from fatty acids and glycerol, like other condensation processes, involves only a negligible energy change.

Phospholipids.—The constitution of these compounds is indicated in a general way by their hydrolytic products which are fatty acids, a nitrogenous base, phosphoric acid, and glycerol. The best known of the phospholipids are the lecithins, which are believed to occur in all plant and animal cells. The widespread occurrence of these compounds suggests that they play fundamental roles in cell metabolism, but it is by no means certain what these are. The following is the probable structural formula of the lecithins, in which R_1 and R_2 represent fatty acid radicals:



Lecithin molecules are similar to fatty acid molecules, the essential difference being that one fatty acid radical is replaced by radicals of phosphoric acid and the nitrogenous base choline. Since various kinds of fatty acid radicals can be combined in the R_1 and R_2 positions as shown in the above structural formula, a number of different lecithins are possible.

Waxes.—These compounds are usually fatty acid esters of saturated monohydroxy (rarely dihydroxy) alcohols such as cetyl alcohol ($C_{16}H_{33}OH$), ceryl alcohol ($C_{26}H_{53}OH$) and myricyl alcohol ($C_{31}H_{63}OH$). Some waxes, however, are fatty acid esters of the sterols (see below). Waxes are of widespread occurrence in both plants and animals. Examples are beeswax, poppy wax, and the wax of the bayberry from which candles are made.

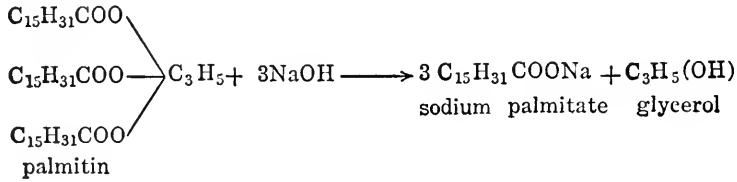
Sterols.—These are complex, cyclic (*i.e.* containing ring groupings) alcohols of high molecular weight. Cholesterol ($C_{27}H_{45}OH$) is the best known of these compounds and is apparently present in all animal cells, being especially abundant in the brain and nervous tissue. Cholesterol is not known to occur in the higher plants, but a number of similar compounds, known as the *phytosterols,* have been isolated from plant tissues. One of the most interesting of the sterols is *ergosterol* ($C_{28}H_{43}OH$). This was first discovered in ergot, but is now known to be widely distributed in plants and animals. It is especially abundant in yeast which serves as its commercial source. Especial interest attaches to this compound, since it is a precursor of the anti-rachitic vitamin D. Upon irradiation ergosterol is converted through a series of intermediate compounds into this vitamin.

Cutin and Suberin.—The chemistry of both of these substances is very imperfectly known, although it has long been recognized that their chemical affinities are with the lipids.

Cutin apparently is a mixture composed principally of free fatty acids (often in oxidized form) and condensation products of the fatty acids such as waxes and soaps. The fatty acids present appear to be preponderantly hydroxy-fatty acids, *i.e.* those which contain one or more hydroxyl groups in the molecule.

Suberin appears to be a mixture of substances consisting principally of condensation products and other modifications of phellonic ($CH_3 \cdot (CH_2)19 \cdot CHOH \cdot COOH$), phloionic ($C_{18}H_{34}O_6$) and other similar acids. The principal distinction between cutin and suberin is that the constituent fatty acids are different in the two materials, and that glycerol is one of the hydrolytic products of suberin, but not of cutin.

Soaps.—Fats react with inorganic bases as illustrated in the following representative reaction:



This reaction is called *saponification* and the resulting salt of the fatty acid, in this example sodium palmitate, a *soap*. Soaps are of common occurrence in plant cells. They are excellent emulsifiers and probably serve in this rôle in the protoplasm.

Rôles of the Lipids in Plants.—Protoplasm always contains lipids in a finely emulsified form. They are especially abundant in the protoplasm of meristematic cells. It is impossible to determine just what proportion of the lipid material dispersed in this way represents storage food which may ultimately be used as such, and how much represents indispensable constituents of the protoplasm. In some cells, particularly those of seeds which are rich in stored fats, relatively large droplets of oil may occur as inclusions in the protoplasm. In the cells of some species, particularly monocots, oil deposition occurs in definitely organized bodies known as *elaioplasts* (Chap. VI).

Fats serve as storage forms of food in plants and this is undoubtedly one of their principal rôles. The fats which accumulate in most plants are liquid within the usually prevailing range of temperatures in temperate zones. In many species, especially conifers, fats may remain in a liquid condition at temperatures as low as -30° C.

Fats are especially abundant as reserve foods in the seeds of many species. Those varieties of seeds in which oils occur in abundance usually contain relatively small quantities of carbohydrates and *vice versa*. Species which produce seeds rich in fats include cotton, corn, peanut, sunflower, rape, flax, and castor bean. All of these species are important commercial sources of vegetable oils. Olive oil, a staple food product in many countries, is extracted from the fruits of the olive. Oils frequently occur in abundance in many other plant organs, as for example in the rhizomes of potato and iris, and in the aerial organs of many woody species, especially during the winter months.

Fats contain larger quantities of energy per unit of dry weight than carbohydrates or proteins, and hence are efficient storage foods. Average values for heats of combustion of 1 g. of dry weight are: fats 9.3 kg.-cal., proteins 5.7 kg.-cal., and carbohydrates 4.1 kg.-cal. The high energy content of the fats is a corollary of the highly reduced state of the carbon in such compounds.

During the germination of fatty seeds the oils present gradually disappear. This is shown for sunflower seeds in Table 37, in which the "ether extract" is taken as a composite measure of the oily constituents present. Concurrently with the disappearance of fats there is a temporary increase in the quantity of soluble carbohydrates present as well as a progressive increase in the amount of cellulose. The carbohydrates are undoubtedly formed from fatty acids and glycerol resulting from the digestion of fats.

It can be shown that fatty acids accumulate temporarily in oily seeds during germination. This cannot be demostrated for glycerol; apparently this compound is transformed into other substances as rapidly as it is produced. The simpler carbohydrates are undoubtedly formed first and cellulose then produced by the condensation of glucose molecules. The protein content of the seeds also decreases during germination but this is probably chiefly due to their conversion into amino acids and acid amides. Subsequently most of the hydrolytic products of the proteins are probably used in the construction of new protoplasm. A part of the carbohydrates resulting from chemical transformations of fats is utilized in the synthesis of cellulose and other cell wall constituents; another portion of them is used in respiration.

TABLE 37—CHANGES IN THE CHEMICAL COMPOSITION OF GERMINATING SUNFLOWER SEEDS, IN TERMS OF GRAMS PER 100 SEEDS OR SEEDLINGS (DATA OF MILLER, 1910)

		Seeds	Seedlings				
			4 days	5 days	7 days	10 days	14 days
Ether extract.....	Cotyledons	3.79	3.00	2.53	1.30	0.50	0.32
	Hyp. and roots	0.22	0.19	0.12	0.21	0.27	0.24
Total sugars.....	Cotyledons	0.28	0.06	0.09	0.12	0.09	0.05
	Hyp. and roots	0.02	0.07	0.30	0.43	0.35	0.16
Reducing sugars....	Cotyledons	....	....	....	0.05	0.09	0.02
	Hyp. and roots	....	0.07	0.27	0.38	0.35	0.16
Protein.....	Cotyledons	1.66	1.18	1.03	0.76	0.63	0.48
	Hyp. and roots	0.12	0.11	0.12	0.20	0.20	0.14
Cellulose.....	Cotyledons	0.15	0.12	0.13	0.18	0.20	0.21
	Hyp. and roots	0.01	0.05	0.10	0.24	0.40	0.32

Similar chemical transformations also occur during the germination of seeds in which foods are stored predominantly in the form of carbohydrates. In such seeds, however, the soluble carbohydrates used in the processes of assimilation and respiration are produced by the hydrolysis of starch and other storage carbohydrates.

The universal occurrence of the lecithins and similar phospholipids in plant cells suggest that they are in some way essential to the maintenance of living protoplasm. According to Jordan and Chibnall (1933) some phospholipids serve as reserve foods. A number of rôles have been ascribed to the lecithins, but there is very little experimental justification for any of the assumptions which have been made regarding their physiological significance. Lecithins are excellent emulsifiers and for this reason have often been assumed to have an important influence on protoplasmic organization and permeability phenomena. Phospholipids seem to have important influences upon the action of oxidase systems in plants and hence are supposed to have effects on the process of respiration. Lecithins are also supposed to have some relation to the formation of oils in plant cells, as described in Chap. XXV.

Essential Oils.—The usual first step in any analysis of plant tissues for lipids is to dissolve them out of the dried and ground tissues with ether. The resulting "ether extract" contains a number of other types of compounds in addition to the lipids. Among these are the *essential oils* and *resins*. These compounds are chemically quite different from the fats, but because of their occurrence in ether extracts it has become customary to discuss them in connection with the fats. Most of these compounds are pungent aromatic substances. They are responsible for many of the distinctive odors and flavors of plants.

The essential oils are not oils at all in the chemical sense of the word. They fall into two groups which are quite distinct chemically. The first of these consists of the *terpenes* which are hydrocarbons of the general formula $C_{10}H_{16}$, of which a large number of isomers exist. A large proportion of the oils of turpentine, lemon, pennyroyal, bergamot, etc., consists of terpenes. Essential oils of the second group all contain oxygen, and some contain sulfur. They are complex alcohols, aldehydes, and ketones, many of them being phenol derivatives. Menthol, camphor oil, thymol, and vanillin (the flavoring principle of the vanilla bean) are examples. Oil of mustard and oil of garlic are sulfur-containing essential oils. The latter is responsible for the distinctive odor and flavor of onions, garlic, and radishes.

The resins are apparently oxidation products of the terpenes, but little is known of their exact chemical constitution. In addition to the hard resins, Canada balsam and crude turpentine are generally classed in this group of substances.

The rôle of the essential oils and resins in plants, if any, is unknown. They are probably by-products of the metabolic activities of the plant.

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CHAPTER XXIV

ABSORPTION OF MINERAL SALTS

The dry matter residue remaining from any plant tissue after desiccation in an oven can be separated by a simple analytical procedure into a combustible fraction, representing organic matter, and an incombustible fraction called the *ash* (Chap. XX). The latter corresponds roughly to the mineral salts which have been absorbed from the soil, but does not include any nitrogen since this element passes off in the combustion process along with carbon, hydrogen and oxygen. The mineral elements do not occur in the ash in the pure state, but mostly as oxides. The actual values obtained for the ash content of a plant tissue depend upon the ignition temperature used. A portion of some of the mineral elements present is often lost by sublimation or vaporization. This is especially likely to happen to chlorine and sulfur, but potassium, calcium, phosphorus, and perhaps other elements are sometimes lost in this way. Hence the ash content of a tissue furnishes only a rather crude measure of the mineral salt content of that tissue.

The rôle of nitrogen in the metabolism of plants is discussed in Chap. XXVI, but from the standpoint of the mechanism of absorption, nitrogen will be included among the mineral elements.

The total ash content of plant tissues and organs varies from a fraction of 1 per cent to 15 per cent or even more of the dry weight of the plant material. Fleshy fruits and woody tissues are usually low in ash content, often yielding less than 1 per cent, while the ash content of leaves is usually relatively high, often exceeding 10 per cent. Tobacco leaves, for example, contain on the average about 12 per cent of ash on a dry weight basis. The ash content of other plant organs usually lies somewhere between these two extremes.

Elements Found in Plants.—It is probable that there is not a single one of the chemical elements which is not found at least in traces in some species of plant, under certain conditions. Actually about 40 of the known elements have been identified as occurring in plants by chemical analysis. The list of these elements includes carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, iron, chlorine, silicon, alu-

minum, manganese, sodium, boron, caesium, lithium, fluorine, rubidium, barium, strontium, bromine, mercury, zinc, tin, lead, thallium, titanium, arsenic, selenium, iodine, chromium, cobalt, vanadium, copper, and silver.

Of this somewhat extensive list only the first fifteen of these elements are found regularly in plants in appreciable quantities and several of these are apparently not essential. On the other hand certain other elements which are seldom present in more than minute traces are indispensable, at least for the continued growth of some species.

The elemental composition of a mature corn (maize) plant is illustrated by the data in Table 38, which may be taken as fairly representative for plants in general.

TABLE 38—ELEMENTAL ANALYSIS OF THE STEM, LEAVES, COB, AND GRAIN OF A MATURE CORN PLANT ("PRIDE OF SALINE"), BASED ON AVERAGE VALUES FOR FIVE PLANTS (DATA OF LATSHAW AND MILLER, 1924)

Element	Weight in grams	Percentage of total dry weight
Carbon	364.19	43.569
Oxygen	371.42	44.431
Hydrogen	52.17	6.244
Nitrogen	12.19	1.459
Sulfur	1.416	0.167
Phosphorus	1.697	0.203
Calcium	1.893	0.227
Potassium	7.679	0.921
Magnesium	1.525	0.179
Iron	0.714	0.083
Manganese	0.269	0.035
Silicon	9.756	1.172
Aluminum	0.894	0.107
Chlorine	1.216	0.143
Undetermined	7.8	0.933

The composition of plant ash varies both with the species and the environmental conditions under which the plant has developed. Comparative figures on the percentages of five of the more important mineral elements in several different species of plants growing in the same soil are given in Table 39.

As the data in this table show, even when growing under identical soil and climatic conditions, different species of plants contain very different proportions of the various elements obtained from the soil. Until the mechanism of the absorption and translocation of mineral elements by plants is

better understood, it is doubtful if any even partially adequate explanation of this fundamentally important fact can be formulated.

TABLE 39—PERCENTAGE OF CALCIUM, POTASSIUM, MAGNESIUM, NITROGEN, AND PHOSPHORUS IN THE TOPS OF SEVERAL SPECIES OF PLANTS GROWN IN A GREENHOUSE IN AN ALBERTA "BLACK BELT" LOAM SOIL (DATA OF NEWTON, 1928)

Species	Percentage of dry weight				
	Ca	K	Mg	N	P
Sunflower	1.68	3.47	0.730	1.47	0.080
Bean	1.46	1.19	0.570	1.48	0.053
Wheat	0.46	4.16	0.225	2.26	0.058
Barley	0.68	4.04	0.292	1.94	0.125

The composition and other properties of the soil in which a plant is rooted will also have an effect on the proportion of each of the various elements absorbed by that plant. Innumerable examples of this fact can be cited from the practice of fertilizing. Addition to the soil of a compound which can be absorbed by plants usually results in an increased intake of that substance by the plants although the increase in the amount of the element within the plant tissues is usually not proportionate to the increase in the amount of that element in the soil. Plants often absorb from the soil mineral salts far in excess of their actual metabolic requirements. Potassium, phosphate, sulfate and other ions often accumulate in plant cells in excess of the quantities actually utilized by the cells.

The Soil as a Source of Mineral Elements.—With only negligible exceptions, all of the mineral elements which enter into the composition of terrestrial plants come from the soil. In discussions of the absorption of both water and mineral salts by plants attention is generally focussed on the soil solution (Chap. XVI). Recent advances in soil science have made it increasingly clear, however, that the mineral salts dissolved in the soil solution are not the only ones which must be considered in any evaluation of the mineral salt relations of soils as they influence the intake of solutes by plants.

The fundamental physico-chemical properties of most soils are due mostly to the clay fraction, except in soils relatively rich in organic compounds in which they also play an important part in determining soil properties. The clay fraction of the soil consists entirely of particles of colloidal dimensions. The clay particles of the soil are apparently composed largely

of alumino-silicates and although of colloidal dimensions possess a definite crystalline structure.

Although it is known that certain uni- and bivalent cations, especially K^+ and Mg^{++}, may occur within the crystalline lattice of the micelles of colloidal clay, from the standpoint of plants a much more important rôle in the ionic relations of soils is played by cations located at the surface of the clay particles. The term "surface" as here used includes the surfaces of the minute capillaries within the micelles as well as their external boundary layer. The micelles of colloidal clay are almost invariably negatively charged, and the cations associated with them may be regarded as occupying a position analogous to the ions in the outer layer of an electrical double layer (Chap. V). The cations most commonly associated with the clay particles of natural soils in this manner are Ca^{++}, Mg^{++}, K^+, Na^+, and H^+. Cations are also similarly associated with the soil colloidal particles of organic origin.

Under certain conditions some of the cations of one kind can be displaced from the micelles by another kind of cation. For example, if a neutral soil is treated with a potassium chloride solution, some of the added K^+ ions replace Ca^{++} ions associated with the clay micelles, an equivalent quantity of Ca^{++} ions being displaced into the solution, pairing off with the residual chloride ions. This reaction is depicted diagrammatically by the following equation:

$$\left[\text{Clay}\right] Ca^{++} + 2\,K^+ + 2\,Cl^- \rightleftarrows \left[\text{Clay}\right]\begin{matrix}K^+\\K^+\end{matrix} + Ca^{++} + 2\,Cl^-$$

Actually each clay micelle may have many cations associated with it, but in the interests of simplicity the reaction has been written in terms of only one adsorbed Ca^{++} ion. Other ions associated with micelles will be displaced in a similar way, although the proportion of any kind of adsorbed ion entering into such exchange reactions will vary greatly depending on the conditions under which the exchange of ions takes place.

The phenomenon which has just been described is called *base exchange* or *cation exchange*. Such interchanges of cations take place very rapidly and are reversible. In neutral and slightly alkaline soils Ca^{++} is the principal replaceable cation, although appreciable quantities of Mg^{++} may sometimes also be present. The H^+ ion is the principal replaceable cation in acid soils, and the Na^+ ion occupies a similar position in alkali soils. All of the exchangeable cations are not retained by the micelles with equal

effectiveness. The order of the retentive capacity of the micelles for cations is $H^+ > Ca^{++} > Mg^{++} > K^+ > NH_4^+ > Na^+$. In other words, of the ions in the above series, the H^+ ions are most tenaciously bound to the colloidal particles and are the most difficult to displace, while the converse is true of the Na^+ ions.

Of the five principal anions found in the soils—Cl^-, SO_4^{--}, NO_3^-, HCO_3^-, and PO_4^{---} —only the last is retained by the soil particles as such in any appreciable quantities. The other four anions usually leach out of soils rather rapidly. Micro-organisms may utilize nitrates and sulfates in the synthesis of organic compounds and thus fix them in the soil in the form of organic nitrogen compounds but this is an entirely different phenomenon from the retention of ions in the soil by a purely physico-chemical mechanism. On the other hand, even in soils which have received heavy treatments of phosphates, only small quantities of PO_4^{---} ions are found in the drainage waters. Evidently the PO_4^{---} ions are tied up by the soil particles in some manner, but the mechanism by which this is accomplished is not entirely understood. It is generally considered that the phosphates are precipitated as insoluble compounds by a reaction with some of the soil constituents, and that in this precipitated form they are not readily available to plants.

The discovery of the phenomenon of cation exchange has made necessary a revision of the older concept of the soil as a rather inert medium containing a soil solution from which plant roots obtain all of the mineral salts which they absorb. Roots not only absorb the freely diffusible ions in the soil solution but can also liberate cations adsorbed on clay micelles which in turn can be absorbed by the plant. In general it appears that the cations which enter plants come largely from the outer layer of the clay micelles while the anions come largely from the soil solution.

The region of the root in which intake of water occurs is also, in general, the zone in which absorption of ions takes place, and the pathway which solutes follow in passing from the soil to the stele is the same as that followed by water (Fig. 70). The root tips are, under favorable conditions, not only rapidly growing organs, but centers of high metabolic activity. Carbon dioxide resulting from respiration is continuously being released into the soil in which it reacts with water, forming carbonic acid. Around each root tip there will usually be, therefore, a localized zone of high carbonic acid content. In this zone H^+ ions from the carbonic acid may displace adsorbed cations on the clay micelles. This is especially likely to happen to the micelles in intimate contact with the root tips. The cations released as a result of this ionic exchange can then be absorbed by the

plant. That plants can release adsorbed Ca^{++} ions from soil colloids in this manner has been demonstrated experimentally (Jenny and Cowan, 1933) and other kinds of ions undoubtedly can be displaced from the clay micelles in the same way. Since roots are more or less continuously growing through the soil they are constantly coming into contact with additional micelles from which cations can be displaced and absorbed. In many soils, in fact, it is only by continuous growth of the roots through the soil that a continued absorption of mineral salts can take place. The rate of root growth is therefore often an important factor both in the absorption of water (Chap. XVII) and the absorption of mineral salts. As a result of ionic exchanges of this type neutral soils tend to become more acid with continuous cropping unless calcium is supplied from time to time.

The application of fertilizers to soils will also usually induce cation exchanges. If an ammonium fertilizer, for example, be added to a soil, some of the NH_4^+ ions will participate in ionic exchanges with cations already adsorbed on the soil particles. This will result in a lower concentration of NH_4^+ ions in the soil solution, but also in an increase in the proportion of certain other ions in the soil solution. Similar cation exchanges almost invariably result whenever other exchangeable cations are introduced into a soil by the addition of fertilizers or in any other way.

The Penetration of Electrolytes into Plant Cells.—The results of most studies on the permeability of the cytoplasmic membranes indicate that electrolytes, being strongly polar compounds, enter plant cells relatively slowly as compared with non-polar compounds (Chap. X). While it is apparently true that the cytoplasmic membranes are relatively less permeable to electrolytes than to non-polar compounds, entrance of electrolytes may, under favorable metabolic conditions within the cells, occur very rapidly. For example, Hoagland and Broyer (1936) record that barley roots may absorb significant quantities of certain ions within two hours. The apparent discrepancy between such results and the results of most determinations of the permeability of cytoplasmic membranes to electrolytes is probably due to the circumstance that most permeability studies have been made with cells in which metabolic conditions favorable to the rapid entrance of electrolytes did not prevail.

It is not known with certainty whether electrolytes penetrate into cells as molecules or as ions. Osterhout (1936) favors the former view. Although electrolytes are largely or entirely dissociated when dissolved in water, considerable evidence indicates that the plasma layers of the cytoplasm are of a lipoidal constitution and electrolytes can dissociate only very slightly when dissolved in such solvents. Other workers, however, believe

that electrolytes pass into cells in the form of ions. Because of electrostatic attraction between oppositely charged ions passage of a cation into a plant cell must be accompanied by passage of an anion or anions of equal electrostatic charge, and *vice versa,* unless the unbalanced electrical forces which would develop as the result of such a situation are compensated for in some other manner. Actually it makes very little real difference in our picture of the kinetics of penetration of electrolytes whether we visualize them entering cells as molecules or as closely associated ions.

The Accumulation of Ions by Plant Cells.—In most elementary discussions of the absorption of mineral salts by plants it has usually been stated or implied that they enter the cells of submerged aquatics or the epidermal cells and root hairs in the absorbing region of roots by a process of simple diffusion from the external medium. The principal implication of such a statement is that the diffusion of an electrolyte into cells takes place as a result of its greater concentration in the external solution than in the cells. The results of a number of investigations indicate, however, that the absorption of electrolytes by plant cells is, under many conditions at least, a much more complicated process than simple diffusion.

That factors other than simple diffusion are involved in the absorption of ions is illustrated by the data in Table 40. These data are based on actual chemical analyses of sap expressed from the cells of the alga, *Nitella clavata,* and of the pond water in which it was growing. As much as a drop of sap can be obtained from a single cell of this species. The samples of sap actually used for analysis generally ranged in volume from 15 to 40 cc.

TABLE 40—ANALYSIS OF THE SAP OF *Nitella clavata* AND OF THE POND WATER IN WHICH IT WAS GROWING (DATA OF HOAGLAND AND DAVIS, 1929)

Ion	Sap concentration (Milliequivalents[1] per L.)	Pond water concentration (Milliequivalents per L.)
Ca^{++}	13.0	1.3
Mg^{++}	10.8	3.0
Na^{+}	49.9	1.2
K^{+}	49.3	0.51
Sum cations	123.0	
Cl^{-}	101.1	1.0
SO_4^{--}	13.0	0.67
$H_2PO_4^{-}$	1.7	0.008
Sum anions	115.8	

[1] A milliequivalent of an ion is one-thousandth its gram ionic weight divided by its valence.

The concentration of every ion analyzed for was greater, and often many times greater, in the cell sap than in the pond water. However, since the cells had been growing continuously in the pond such marked differences in concentration probably represent an equilibrium condition between the cells and the pond water. In other species it has been shown that, under some conditions at least, the NO_3^- ion may also accumulate within cells in greater concentration than in the outside medium. The total concentration of electrolytes in the cell sap of *Nitella* was about twenty-five times as great as in the pond water which bathed the cells.

Formerly it was generally assumed that the accumulation of ions within plant cells is possible because they are adsorbed, precipitated, or otherwise rendered kinetically inactive. Evidence from conductivity measurements (Hoagland and Davis, 1923) indicates very strongly that an overwhelmingly large proportion (probably at least 90 per cent) of the inorganic elements in the cell sap of *Nitella* are present in the ionic state and are not tied up in some non-dissociated form. The total concentration of the cations in the sap of this species exceeds the total concentration of anions, although not greatly. Most of the excess cations are probably paired off with the anions of organic acids.

An increase in the concentration of the free ions in the cell sap to a value many times greater than their concentration in the external medium can only be attained as a result of the diffusion of those species of ions against a concentration gradient, *i.e.* from a region of the lesser concentration of each individual ion to a region of its greater concentration. A number of other species of submerged aquatic plants such as *Valonia, Elodea, Halicystis,* and *Chara* apparently possess a similar capacity of accumulating electrolytes (Osterhout, 1936). In some of these species, notably *Valonia,* it also seems to have been demonstrated beyond question that the ions are present in the cell sap in the free state.

Accumulation of electrolytes also occurs in some of the tissues of terrestrial plants. Steward (1932, 1933) has obtained convincing evidence of a correlation between the rate of aerobic respiration (Chap. XXIX) and the accumulation of electrolytes by potato tuber tissue. When thin disks cut from a potato tuber were immersed in dilute solutions of potassium bromide it was found that accumulation of K^+ and Br^- ions occurred only when the solution is suitably aerated, the two ions often, but not always, being absorbed in approximately equivalent amounts. Within limits absorption of Br^- ion closely paralleled the percentage of oxygen in the air used to aerate the solution (Fig. 95). The absorbed ions were found to reside chiefly in the superficial layers of cells of the disks of potato tuber tissue in-

dicating that increased respiration of those cells, due to adequate aeration, was causally connected with the accumulation of ions. Concurrently with the absorption of ions a marked diminution occurred in the starch content of the peripheral layers of cells. Presumably some or all of the glucose resulting from the digestion of starch was oxidized in respiration.

That the absorption of ions by roots is also influenced by aeration of the circumambient solution has been shown by Hoagland and Broyer (1936). In one experiment excised roots of barley plants were transferred to a solution in which potassium bromide was present in a concentration of 0.0075 M and calcium nitrate in a concentration of 0.0025 M. When a stream of air was passed through the solution K^+, Br^-, and NO_3^- ions accumulated rapidly in the cells. When a similar experiment was performed in which a stream of nitrogen was passed through the solution instead of air very little absorption of ions occurred. Evidently a high rate of aerobic respiration is correlated with the cellular activities which bring about the accumulation of both cations and anions in root cells. The sugar content of the roots decreased during rapid aeration, and if the roots were first depleted of sugars their capacity for the absorption of electrolytes was greatly diminished. Other experiments by the same workers indicated that not only is a relatively high rate of aerobic respiration required for marked accumulation of ions, but the ions already present in the root cells may not be retained if the rate of respiration falls too low. Low temperatures were also found to result in a marked retardation in the rate of accumulation of ions, presumably because of the correlated decrease in the rate of respiration.

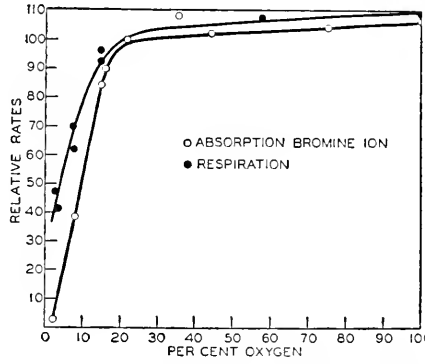

FIG. 95. Relation between oxygen content of the solution, relative respiration and relative absorption of bromine ions. Figures on abscissa represent per cent of oxygen in the oxygen-nitrogen mixtures used to aerate the solution. Data of Steward (1933).

Prevot and Steward (1936) have demonstrated the presence of a longitudinal gradient of accumulation in barley roots extending from the apex to the region of emergence of the secondary roots. Accumulation of Br^- ions was found to be greatest in the apical 1 cm. portion and to decrease progressively with increasing distance from the tip. The same is presumably true for other ions. This gradation in the accumulative capacity of the

root cells is directly correlated with the progressive diminution in metabolic activity of the cortical cells of the root with increasing distance from the apex. Under the conditions of these experiments most of the accumulation of ions occurred in the cortical cells.

Not only do ions accumulate in the cortical cells of a root from an external solution, but there is some evidence that the cells of the stele (tissues internal to the endodermis) accumulate ions from the cortical root cells (Steward, 1935).

That there is a close correlation between the accumulation of ions by plant cells and their rate of respiration seems to have been demonstrated beyond doubt. This has been shown for cells from a number of very diverse plant tissues. Three conditions known to be necessary for the occurrence of this phenomenon are apparently essential because they contribute to relatively high rates of aerobic respiration. These are: (1) an adequate supply of oxygen, (2) a suitable temperature, and (3) a supply of sugars or of easily hydrolyzable polysaccharides within the cells.

In *Valonia* and *Nitella* there seems little doubt that the ions occur in the cell sap in the free state. It is generally assumed that this is also true in the cells of the higher plants although the evidence for this is largely presumptive. If this is granted it follows that the ions move into such cells in opposition to a concentration gradient. Theoretically the movement of ions into a plant cell in opposition to a concentration gradient would require energy. Likewise the retention of ions within cells in greater concentration than in the external medium also requires a continuous expenditure of energy if the cell membranes remain permeable to those ions. The relation between respiration and salt accumulation is certainly not a simple one, but a partial explanation may be that energy of respiration is utilized in the accumulation and retention of ions.

It has also been found that rapid accumulation of an ion does not take place in cells in which the concentration of that ion is already relatively high. The previous metabolic history of the cells, particularly as regards electrolytes, will therefore have an important influence upon the capacity of those cells for further accumulation of electrolytes.

The phenomenon of accumulation of salts at appreciable rates seems to be confined largely if not entirely to cells which have not yet lost the capacity for cell division and growth (Berry and Steward, 1934). There are some indications that meristematic cells possess the most marked capacity for accumulation, and that with a decrease in the proportion of growing cells or of cells capable of renewed growth in any tissue, its capacity for the accumulation of electrolytes diminishes, even under conditions which are

otherwise entirely favorable for the process. For example the parenchyma tissue of apple and pear fruits is fully mature and shows no accumulation, while disks cut from potato tubers and many other similar storage organs are capable of renewed growth and exhibit rapid accumulation under favorable conditions. The most rapid rates of accumulation yet discovered have been found in the apical region of young roots. It is possible that all or most plant cells possess this capacity when in a meristematic condition, but that it is lost by many as they grow older.

Certain non-electrolytes will also accumulate in plant cells. It has long been known that plant cells will continue to absorb certain dyes (methylene blue, neutral red, etc.) until their vacuolar concentration far exceeds that in the outside medium. It is doubtful, however, whether the mechanism of dye accumulation is the same as that operating in the case of electrolytes. Evidence is also gradually accumulating that sugars may also move into certain plant cells against a concentration gradient and ultimately it may be found that plant cells can accumulate other kinds of organic compounds.

Ionic Exchanges between Cells and the External Solution.—Brief reference should be made to another type of phenomenon which often has an influence on the mineral salt composition of the cell sap of plant cells. The accumulation of salts, as previous discussion has shown, seems to occur only in actively metabolizing cells. In this process both anions and cations are absorbed, usually in approximately equivalent quantities. Steward (1935) has suggested that this process be called "primary salt absorption" in order to distinguish it from other processes which also influence the ionic composition of the cell sap.

Exchange of ions between the cell sap and a circumambient solution has been observed in a number of different plant tissues. This process appears to be more characteristic of mature cells which have ceased to accumulate salts, although it also occurs in younger, metabolically active cells. The simplest mechanism which can be postulated to account for such ionic exchanges is that the ion which diffuses into the cell sap is compensated for by diffusion out of the cell by one or more ions of another kind, but of equal electrostatic value. An example of such ionic interchange between cells and an external medium is the previously cited exchange of H^+ ions from root cells for other cations in the soil. Such an exchange mechanism will explain the apparent capacity of cells under certain conditions to absorb cations or anions without their satellite ions of opposite charge. Even if it is assumed that the electrolytes cross the plasma membranes in the form of molecules rather than as ions, essentially the same picture of the process is adequate. It is only necessary to assume that both the outgoing and incoming cations

are accompanied by anions of the same kind as they cross the plasma membranes, or *vice versa,* if the interchanging ions are anions.

Such ionic exchanges can only take place if the cells already contain dissolved electrolytes which have been obtained by primary absorption or by translocation into them from other cells.

The intake of cations or anions by plant cells may sometimes involve an exchange of ions between water and an electrolyte in the external solution before inward diffusion occurs. The ions which pass into the cell may be accompanied by hydrogen ions (if anions) or by hydroxyl ions (if cations) of equal electrostatic value, leaving their erstwhile partners compensating hydrogen or hydroxyl ions. For example, the K^+ ion of KCl might diffuse into a cell unaccompanied by a companion Cl^- ion, provided it were attended in its passage by an OH^- ion. The deserted Cl^- ion would then pair off with the original escort of the OH^- ion, a H^+ ion originating from the dissociation of a water molecule. Such a mechanism would result in an increase in the hydrogen ion concentration of the solution. Similarly penetration of anions accompanied by hydrogen ions into plant cells would result in a decrease in the hydrogen ion concentration of the external solution. Such changes in the pH of the circumambient solution have sometimes been observed upon the entrance of cations or anions into plant cells. Such a mechanism will work whether the anions and cations are visualized as crossing the cytoplasmic membranes as molecules or as closely associated ions.

Since water almost invariably contains dissolved carbonic acid it is also probable that the H^+ and HCO_3^- ions of this compound may act in a manner analogous to the H^+ and OH^- ions of water in facilitating the entry of anions and cations respectively into plant cells.

Absorption of Mineral Salts by Aerial Organs.—In nature absorption of mineral salts through the aerial organs of the plant rarely occurs. Plants are sometimes "fertilized," however, by direct treatment of aerial organs, which involves the absorption of the added compounds through the leaves or stems. For example, such a practice is followed in treating iron-deficient pineapple plants in Hawaii. The plants are sprayed with a dilute solution of ferrous sulfate, enough of which is absorbed directly through the leaf surfaces to remedy the chlorotic condition which has developed as a result of iron deficiency. Similarly manganese deficiency has been corrected in some species by spraying the plants affected with a dilute solution of a manganese salt.

Discussion Questions

1. Suggest as many possible reasons as you can for the fact that different kinds of plants, growing in the same soil and under the same climatic conditions, absorb various ions in different proportions.
2. Can the mineral element requirements of a plant be judged from a chemical analysis of the plant?
3. What are the soil factors which will influence the rate of absorption of ions by the roots of plants?
4. When plants are grown with their roots in a solution containing calcium nitrate the H-ion concentration of the solution usually shows a gradual decrease. If ammonium sulfate is used as a source of nitrogen, however, the solution usually increases in H-ion concentration. Explain.
5. How would you attempt to show experimentally whether or not absorption of water and mineral salts by plants are largely independent processes?

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CHAPTER XXV

UTILIZATION OF MINERAL SALTS

A clear distinction should be drawn between the *absorption* of a salt and the subsequent *utilization* of it or its component ions. The term utilization is employed in a loose sense to refer to the incorporation of mineral elements into the relatively permanent constituents of the cell walls and protoplasm. Absorption of the ions or molecules of salts does not necessarily mean that they will be utilized. Many of the ions absorbed by a plant remain for more or less indefinite periods in the ionic state in the cells. Sooner or later many of these ions are usually incorporated either into the structure of more complex but unassimilated molecules synthesized by the plant such as storage proteins, calcium oxalate, glycosides, etc., or into the protoplasm or cell walls proper. There may, therefore, be a considerable time lag between the absorption of an ion and its utilization, while some of the absorbed ions may remain indefinitely as such within the cells. Furthermore, some mineral elements may be utilized in one organ of a plant, subsequently released by disintegration of cell constituents, translocated to other organs of the plant, and there reutilized. Redistribution of minerals which have accumulated in cells but have not actually been utilized is also of common occurrence in plants.

General Rôles of Mineral Elements in Plants.—Considered as one group or class of substances found in plants, the mineral elements function in a number of distinct ways:

1. *Building Material from Which Parts of Protoplasm and Cell Walls Are Constructed.*—A number of the mineral elements become permanent parts of molecules which are integral parts of the protoplasm and cell walls. As examples we may cite the sulfur in proteins generally, the phosphorus in nucleo-proteins and lecithins, the magnesium in chlorophyll, the calcium in calcium pectate, etc. Uncritical discussions of mineral elements in plants often consider this to be the sole rôle of these elements. Actually a very large proportion of the mineral elements in plants act in some other way than as material from which essential parts of plant cells are constructed, or else are of no apparent consequence in the metabolism of the plant whatsoever.

2. *Influence on the Osmotic Pressure of Plant Cells.*—In Chap. VIII it was shown that a portion of the osmotic pressure of the cell sap of any plant cell is due to the dissolved mineral salts which it contains. While in most plant cells the absolute concentration of mineral salts in the plant sap is so low that only a small proportion of the osmotic pressure can be ascribed in their presence, there are some important exceptions to this statement as shown previously.

3. *Influence on Acidity and Buffer Action.*—The mineral salts absorbed from the soil often have an influence on the pH of the cell sap and other parts of plant cells, although usually not a very great one, as organic acids and other compounds resulting from the metabolic activities of plants usually exert the predominant influence in determining pH values within cells. As shown in Chap. VI, two of the important buffer systems found in plants—the phosphate and the carbonate systems—have their origin in substances absorbed by the plant from its environment. The phosphate system, however, is the only one found in plants which may properly be classed as a mineral element buffer system.

4. *Influence on the Hydration of Cell Colloids.*—The effects of cations upon the hydration of colloidal materials has already been discussed in Chap. IX. Anions also have an effect upon the hydration of colloidal micelles, but in most organic colloids their effect is less marked than that of cations. The cations and anions in plant cells undoubtedly influence the degree of hydration of the colloidal micelles in the protoplasm and other parts of plant cells.

5. *Influence on the Permeability of Membranes.*—As already discussed in Chap. X the permeability of the cytoplasmic membranes is markedly influenced by the cations and anions in the medium with which they are in contact.

6. *Toxic Effects of Mineral Elements.*—Many mineral elements, in their ionic form, have a decided toxic effect upon protoplasm, often resulting in its disorganization and death. This is especially true of salts of the "heavy metals" such as iron, copper, mercury, etc. Even some of the elements most essential for the existence of plants such as magnesium, calcium and potassium may exert at least a slight toxic effect under certain conditions. This is shown by the fact that plants grown with their roots immersed in dilute solutions of almost any salt will usually die sooner than similar plants with their roots immersed in distilled water. The toxicity of any element varies greatly depending upon its ionic concentration. Further examples of the toxic effect of ions are mentioned later in this chapter.

7. *Antagonistic Effects.*—The antagonism between univalent and bivalent cations in effects upon permeability has already been considered in Chap. X.

Similar antagonistic effects are evident in toxicity phenomena. For example in one experiment it was found that the roots of lupines would elongate only about 3.5 mm. per day in a solution of about 0.000015 M $CuCl_2$, but if sufficient $CaCl_2$ were added to make its concentration in the solution about 0.0078 M the roots elongated at a rate of 10.5 mm. per day. The antagonism between the Cu^{++} and Ca^{++} ions was sufficient to reduce greatly the toxicity of the Cu^{++} ions.

8. *Catalytic Effects.*—Certain effects of mineral elements in plants have generally been interpreted as due to their action as catalysts. Iron and manganese, for example, are considered to act in a catalytic or regulatory rôle in the synthesis of chlorophyll. The action of some mineral compounds as coenzymes (Chap. XXVII) may also be an example of such effects.

Foods or Raw Materials?—As with most of the other terms used in the biological sciences a rigid definition of the word "food" is impossible. The statement that a food is any substance which can be utilized directly by a living organism as building material and also as a source of energy (upon oxidation) probably comes as close to a satisfactory definition of a food as is possible. It is commonly considered that carbohydrates, fats, and proteins are the most important foods of animals. Less generally is it recognized that compounds of these same three classes are also the most important foods used by green plants. The fundamental difference between the two types of organisms is that animals ingest carbohydrates, fats, and proteins from the outside environment, while green plants synthesize them from a small number of relatively simple compounds which they obtain from their surroundings. Failure to appreciate this fundamental point regarding plant metabolism has contributed to the persistence, especially in the popular mind, of the long disproved idea that plants obtain their food from the soil.

Those compounds which enter a green plant from its environment and which cannot in any critical sense of the word be considered foods are most conveniently termed *raw materials.* The raw materials used by green plants include, in addition to the necessary mineral salts, the carbon dioxide and water used in photosynthesis. The compounds classified as raw materials are not only utilized in the synthesis of foods but function in the plant in many other ways. This is true not only of the mineral salts, as the immediately preceding discussion has shown, but of water and carbon dioxide as well.

Essential and Non-essential Elements.—Of the large number of elements which have been identified as occurring in plant tissues, only a limited number have been found to be indispensable. Beginning about 1860 a number of extensive investigations were undertaken to determine specifically which elements are essential for plants, and which are not. Some of the earliest work-

ers on this problem were the German botanists, Sachs and Knop. Their investigations, conducted by the method of solution cultures (see later) and subsequently confirmed by a number of other workers, indicated that in addition to the elements carbon, hydrogen, and oxygen, obtained by plants from water or from atmospheric gases, the only essential elements were nitrogen, phosphorus, sulfur, calcium, magnesium, potassium, and iron, all of which enter the plant from the soil.

From the work of these investigators and others developed the almost classical precept that ten elements, and ten only, were essential for the existence of green plants. This viewpoint was first seriously challenged by Mazé (1915) who considered that at least several other elements are essential for the continued development of green plants. The older concept of the "ten essential elements" was so generally accepted, however, that Mazé's contentions evoked very little immediate interest. Only in comparatively recent years have extensive studies been undertaken on the problem of the possible rôles of other elements in plant metabolism.

It is now realized, however, that there were certain unrecognized sources of error in the experiments upon which the conclusions of earlier investigators were based. Almost all of their investigations were pursued by the method of solution cultures, in which "pure" chemicals in certain proportions were dissolved in distilled water, and these solutions were used as the medium in which the plants were rooted. Many of the "pure" chemicals used, however, contained at least traces of other compounds which might be sufficient in amount to supply plants with an adequate quota of certain necessary elements, especially if they were required only in minute quantities. Similarly, the elements stored in the seed were not usually considered in such experiments. The amounts of some elements available to a plant from this source might suffice for its entire life history if they were only required in small quantities. Furthermore, it has also been more generally realized in recent years that small amounts of certain elements often dissolve in solution cultures from the walls of the containers and thus become available for utilization by plants. Traces of silicon and zinc, for example, may dissolve out of the walls of ordinary glass vessels into solutions contained within them. Even distilled water, of the grade generally used in such experiments, may contain amounts of elements required only in traces sufficient to supply the needs of the plants. For these reasons, therefore, it is clear that in practically all of the earlier experiments designed to determine which elements are essential for plants, small quantities of various elements other than those deliberately supplied were usually present in the solution cultures. The failure of earlier investigators to recognize the possibility of the presence of such contaminating

substances makes it impossible to accept the results of their investigations as the final word on the mineral salt requirements of plants.

Recognition of these sources of contamination in solution culture technique has led, in recent years, to refinements in such methods, which eliminate or at least enormously reduce the possibility of introducing unknown solutes into solutions used in plant culture work. By repeated crystallizations it is possible to obtain chemicals of a much higher degree of purity than those used by the earlier workers. The water used may be distilled from special stills and re-distilled a number of times to remove even the smallest traces of most solutes. Containers can be used which are inert when in contact with the culture solutions employed. By removal of the cotyledons at a very early stage in germination, or by other procedures, the supply of elements obtained by the plant from the seed can be largely eliminated.

Various more or less critical observations have led to claims that many other elements are essential for normal plant development in addition to the ten which have long been accepted as essential to plants. Among these are arsenic, aluminum, barium, boron, bromine, caesium, chromium, chlorine, cobalt, copper, iodine, lithium, manganese, nickel, selenium, silicon, strontium, tin, titanium, vanadium and zinc. Most of these elements are considered to be necessary for plants only in traces. With only a few exceptions all of them, in fact, are toxic in any appreciable concentration.

In the face of this rather overwhelming array of possibly essential elements it appears desirable to adopt a criterion by which the indispensability of an element can be judged. While it is undoubtedly true that any one of these elements, at least when supplied to certain species of plants, under certain cultural conditions, will result in beneficial effects upon growth, this is far from indubitable evidence of its indispensability. Necessity of an element for normal plant metabolism is demonstrated only if lack of it can be shown to result in injury, abnormal development, or death of plants when grown in sand or solution cultures by a technique including the refinements of method described above. Complete proof of the necessity of an element also requires demonstration that no other element of similar properties can be substituted for it. Furthermore, before it can be considered to be proved that a given element is essential for green plants generally its indispensability must have been demonstrated for a wide enough variety of species that a cross section of the plant kingdom is represented.

At the present time practically all botanists agree that at least two of the elements in the list just cited—boron and manganese—must be added to the group of elements essential in plant metabolism. In addition evidence for the indispensability of the two others—copper and zinc—is believed by many in-

vestigators to be convincing enough to warrant their addition to the list of certainly necessary elements. Future investigations may eventually result in further additions to the list of essential elements.

Specific Rôles of the Mineral Elements in Plants.—Although it is conventional to speak of the "ash" and "non-ash" elements in plants, from the standpoint of plant metabolism this is a purely artificial distinction. Of the non-ash elements the rôles of carbon, hydrogen, and oxygen in the synthesis of carbohydrates, fats, and related compounds have already been discussed. There are few organic compounds of physiological importance in either plants or animals which do not contain all three of these elements. The rôle of nitrogen, the other non-ash element, in the synthesis of proteins and other important nitrogen-containing compounds will be considered in the following chapter. Each of the essential mineral elements is known to play certain specific rôles in plants not to be entirely described in terms of the general functions of mineral elements which have already been summarized.

The parts played by the mineral elements in plant metabolism are incompletely understood and it is probable that each of them is involved in metabolic and physico-chemical processes occurring in plant cells in ways which are not at present recognized. When an element is a constituent of some important plant compound—such as the sulfur in proteins, or the magnesium in chlorophyll—that particular rôle of the element is relatively easy to identify. When, however, an element is a necessary link in one of the many complex chain reactions occurring in plant cells, its exact rôle is usually obscure and is often difficult to recognize.

Sulfur.—As a rule this element seems to be fairly well distributed throughout the organs and tissues of the plant. Sulfur is a constituent of the amino acid *cystine* which in turn is one of the compounds from which plant proteins are made (Chap. XXVI). It is also a constituent of *glutathione,* a compound which is supposed by many investigators to play a fundamental part in respiration processes (Chap. XXX) and of the mustard oil glycosides such as *sinigrin,* which impart characteristic odors and flavors to such species as mustards, onions, and garlic. Much of the sulfur present in plants often remains in the inorganic form as sulfates. Most of the sulfur in the organic molecules present in plants occurs in reduced forms. Sulfur which has accumulated in one organ of a plant may subsequently be redistributed to other organs.

The presence of an abundance of sulfur in the soil apparently favors root formation in many species. Chlorophyll development is often retarded in sulfur deficient plants. The pale green color of such plants soon changes to a deep green when sulfur is applied. An abundance of sulfur also favors

root nodule development in legumes, while a deficiency of this element has been reported to have a retarding effect on cell division and fruiting in some species.

Phosphorus.—A very large proportion (often over 50 per cent) of the phosphorus in a mature plant is located in the fruits and the seeds. Phosphorus apparently is readily redistributed in plants from one organ to another. Young growing tissues, therefore, may gain in phosphorus content at the expense of older tissues. Such redistributions are more likely to occur when the supply of phosphorus in the soil is somewhat deficient.

Unlike nitrogen and sulfur, phosphorus is not reduced in plants but is linked into organic combinations in a highly oxidized form. Phosphorus enters into the composition of *lecithin* and other phospholipids and of the *nucleic acids*. The nucleoproteins are synthesized by chemical combination of nucleic acids with proteins (Chap. XXVI). Deficiency of phosphorus interferes with the synthesis of these compounds and hence may interrupt normal cell division in meristematic tissues. Phosphates act as the co-enzyme of zymase (Chap. XXX) and seem to exert an accelerating effect on other oxidizing and reducing enzymes. There is also some evidence that phosphorus is necessary for hydrolytic transformations of carbohydrates in plants, particularly the change from starch to sugars.

In general phosphorus is most abundant in young, meristematic cells, and is utilized in considerable quantities in such growing regions in the formation of nucleoproteins and other phosphorus-containing compounds. Phosphorus is also used in considerable quantities during the period of maturation of fruits and seeds. The phosphorus requirements of annual plants are therefore relatively heavy during the first few weeks after germination, and again near the end of their life cycle.

Addition of phosphates to the soil often favors root development. Hence soil treatment with phosphates is a standard agricultural practice in growing root crops such as turnips and mangolds.

Calcium.—A large proportion of the calcium in most plants is located in the leaves. Calcium is relatively immobile in plants, little redistribution of this element occurring from tissues in which it has once been utilized to other parts of the plant.

Calcium apparently plays a manifold rôle in plant metabolism. It is a structural component of plant cell walls in the form of the calcium pectate of the middle lamella. Calcium ions have pronounced effects upon the permeability of the cytoplasmic membranes and upon the hydration of colloids. Deficiency of calcium results in a stunting of root development of many species. There is also some evidence that an insufficient supply of calcium

interferes with the translocation of carbohydrates and amino acids. Calcium is of widespread occurrence in plants in the form of calcium soaps and is believed by some investigators to occur in plant cells as calcium proteinates. Calcium is of frequent occurrence in plant cells in the form of insoluble crystals of calcium oxalate, and also forms salts by reactions with other organic acids. Some investigators consider that calcium performs an important function in combining with the organic acids which are by-products of protein synthesis, thus preventing the accumulation of organic acids within the cells.

Magnesium.—This element is the one and only mineral constituent of the chlorophyll molecule. A large proportion of the magnesium present in plants is therefore in the chlorophyll-bearing organs, although seeds are also relatively rich in this element. Magnesium generally occurs in soils in sufficient abundance to supply the needs of plants, although occasional exceptions to this statement are found. Deficiency of magnesium results in the development of a characteristic chlorosis (Table 41). Redistribution of magnesium from older to younger organs of plants occurs readily.

Magnesium is believed by some workers to be intimately related to oil formation and the synthesis of nucleo-proteins in plant cells. It has been suggested that the rôle of magnesium in these processes is that of a carrier of phosphates. Phosphates are used in the synthesis of the nucleic acids, one of the constituents of the nucleo-proteins. Phosphates also enter into the composition of *lecithin*. The formation of oil appears to be preceded or accompanied by the synthesis of this compound. Filaments of the alga *Vaucheria* fail to develop oil droplets when growing in media in which magnesium is lacking. Magnesium is much more abundant in oily than in non-oily seeds.

Excess quantities of magnesium may prove toxic in solution cultures, an effect which may be offset by the presence of sufficient quantities of calcium (antagonism!). It is doubtful, however, if magnesium ever exerts such toxic effects in soils.

Potassium.—The bulk of the potassium absorbed by a plant ordinarily moves into it in the earlier stages of its life history. Unlike all of the other ash constituents required by plants in appreciable amounts potassium is not definitely known to be built into organic compounds of fundamental physiological significance. It occurs in plants almost solely as soluble inorganic salts. Potassium salts of organic acids also occur in plant cells. In spite of this fact potassium is an indispensable element and cannot be completely replaced even by such chemically similar elements as sodium or lithium. The young and active regions of plants, especially buds, young leaves, root tips, etc. are always rich in potassium. Older tissues in which relatively few living cells remain—such as wood—contain relatively little potassium. The

proportion of potassium in seeds is also low. Internal redistributions of this element occur readily and more or less continuously during the life history of a plant. Older leaves and other organs frequently lose potassium which is transported to growing regions.

The exact rôle of potassium in plants is obscure. Since it apparently is not used in the construction of any vitally necessary cell constituents its rôle is undoubtedly to be interpreted as chiefly a regulatory or catalytic one. It may exert many of its effects by influencing enzymatic activity. Certain results of a deficiency of potassium upon plant metabolism have long been recognized. While the evidence for none of these effects can be regarded as incontrovertible, potassium appears to be necessary for the normal maintenance of the following processes: (1) the synthesis of simple sugars and starch, (2) translocation of carbohydrates, (3) reduction of nitrates, (4) synthesis of proteins, particularly in meristems, and (5) normal cell division. This last effect is probably closely connected with the rôle of this element in the synthesis of proteins. In the absence of potassium the cells elongate, but do not divide.

The potassium ion is usually the most abundant univalent cation in plant cells, and undoubtedly exerts important effects upon such phenomena as the permeability of the cytoplasmic membranes, hydration of the protoplasm, etc.

Iron.—A deficiency of available iron in soils is seldom a limiting factor in plant development, although occasional exceptions to this statement are encountered. Deficiency of iron in soils is usually due to its insolubility rather than to its actual absence. In general a larger proportion of the iron is in a soluble state in relatively acid soils than in approximately neutral or alkaline soils.

Iron is indispensable for the synthesis of chlorophyll in green plants. Deficiency of this element results in the development of a characteristic chlorosis (Table 41). Iron does not, however, enter into the constitution of the chlorophyll molecule. The *state* of the iron in plant tissues is also often a factor determining its influence in chlorophyll synthesis. Chlorosis due to iron deficiency is sometimes found in plants which contain considerable quantities of iron (Rogers and Shive, 1932). In such plants the iron is present in a precipitated or otherwise unavailable form.

Iron is also supposed to act as a catalyst or oxygen-carrier in oxidation-reduction processes occurring in living cells. Living protoplasm contains traces of organically bound iron which may function in this way.

Iron salts in any appreciable concentration are toxic to plants; for equal concentrations ferrous salts are generally more toxic than ferric salts. While toxic effects of iron can readily be demonstrated in solution cultures, it is

doubtful if iron toxicity occurs in natural soils except possibly under conditions of extreme acidity, or serious lack of aeration, or both.

The proportionate amount of iron in plant tissues is very low; much of that present is a constituent of organic compounds. Iron is one of the most immobile of all elements within plants, no appreciable redistribution ever occurring from one tissue to another. If plants which have been supplied with iron are transferred to a solution culture lacking this element the subsequently developing leaves exhibit a marked iron chlorosis, while the older leaves retain their normal green color. This is a graphic demonstration of the fact that no appreciable transfer of iron occurs from the older to the younger leaves.

Boron.—Recent investigations (Sommer and Lipman 1926, Brenchley and Warington 1927, McMurtrey 1929, Johnston and Fisher 1930, McHargue and Calfee 1933, and others) have demonstrated convincingly that boron is essential for a number of species of plants although it is required only in minute quantities. Species for which this element has been shown to be necessary include beans, barley, buckwheat, melons, mustard, flax, castor bean, cotton, tomato, tobacco, sunflower, peas, sugar beet, sugar cane, citrus fruits, and lettuce. In all probability boron is an essential element for all species of green plants. The exact rôle of this element in plants is unknown, but it is noteworthy that its absence usually affects the meristematic tissues, causing blackening and death or growth abnormalities. In any appreciable concentration boron is toxic to plants.

Manganese.—A number of recent investigations (McHargue and Calfee 1932, Haas 1932, Clark 1933, Hopkins 1934) as well as some earlier ones, indicate conclusively that this element is necessary for a number of species of plants, and manganese is now generally considered a necessary element for all green plants. Manganese is, as a rule, most abundant in the physiologically active parts of plants. It is supposed to play a part in oxidation and reduction phenomena, perhaps largely or entirely through its influence on the activity of oxidase enzymes (Chap. XXX). In fact some investigators think that oxidases may be manganese compounds. Others hold that manganese compounds serve as co-enzymes of the oxidases. Manganese is also related in some way to chlorophyll synthesis, as a deficiency of this element usually results in a development of a chlorotic condition in plants. Chlorosis due to manganese deficiency is different from chlorosis resulting from lack of iron or magnesium (Table 41). Except in very low concentrations manganese compounds are distinctly toxic to plants.

Copper.—This element is widely distributed in plants although it never constitutes a large proportion of the ash. In general copper is highly toxic

to plants except in very dilute concentrations. A number of observations are on record indicating that copper salts may have a beneficial effect upon plants under cultural conditions. The productivity of peat soils in particular can usually be increased by applications of copper sulfate. According to Sommer (1931), copper can be shown to be essential for flax, tomato, and sunflower plants by a solution culture technique. Similarly Lipman and MacKinney (1931) seem to have demonstrated its indispensability for barley and flax plants. Although critical experimental results are still too scanty to permit a general conclusion, indications are that copper will prove to be one of the essential elements for plants.

Zinc.—A number of earlier investigations indicated that this element has a beneficial effect upon plants. More recently Sommer and Lipman (1926) have apparently demonstrated the necessity of zinc for sunflower and barley by a highly refined solution culture technique. This element also seems to be necessary for buckwheat, Windsor beans, and red kidney beans (Sommer, 1928). The quantity of zinc required is, as would be expected, exceedingly minute, and in any appreciable concentrations this element is highly toxic. There is a strong presumption, therefore, that zinc, like copper, will prove to be an essential element for many species of higher plants.

The remainder of the elements to be discussed in this section are not generally considered to be essential although some of them exert marked effects on the development or metabolism of plants.

Sodium.—This element practically always occurs in the ash of plants, but does not seem to be one of the essential elements. In some halophytes it is present in considerable amounts, most of it being dissolved in the cell sap in the form of sodium chloride. In part sodium can replace potassium as one of the essential elements, but no plant will survive in the total absence of potassium, even if sodium is available.

Silicon.—This element comprises a very large proportion of the ash of some species, particularly of the aerial portions of members of the grass and *Equisetum* families. It is also relatively abundant in the bark of trees. Earlier investigators believed, principally because of the large amounts present in the ash of many species, that silicon is essential for plants. Many years ago, however, it was shown that even those species in which silicon was most abundant could be grown to maturity in culture solutions to which no silicon was added. It is doubtful, however, if plants have ever been grown in the complete absence of this element since even in cultures to which it is not supplied traces are probably present in the form of impurities from various sources. There is therefore a possibility that minute traces of this element may be necessary. Formerly it was believed that silicon was important in

contributing to the stiffness of the straw in cereal crops, but more recent experiments do not support this view. Some investigators have also considered that the silicified cell walls of some species, such as the cereals, render them more resistant to the attacks of fungous and insect parasites.

Silicon appears, however, to exert an important influence upon the phosphate metabolism of plants. Application of sodium silicate results in an increase in the yields of plants growing in plots inadequately supplied with phosphates. Some workers believe this effect of silicon to be upon the metabolism of the plant itself. They believe that the presence of this element in some way increases the efficiency of the plant in the utilization of phosphorus. Others believe that silicon increases the availability of the phosphates in the soil. It is possible that both such effects may occur.

Chlorine.—This element was earlier considered to be essential for plants, although in more recent times this view has been abandoned. Chlorine seems, however, to be of universal occurrence in plants but is apparently present almost wholly in the form of inorganic chlorides. Chlorine is a constituent, however, of the molecules of anthocyanins (Chap. XXII). The experimental results which have been obtained upon supplying chlorides to plants have been very variable. In some species a definite beneficial effect has been noticed, in others applications of chlorides have resulted in a retardation of plant growth, while in still others no apparent influence could be detected. The apparent results of supplying chlorides to plants may be largely if not entirely due to changed ionic relationships in the soil or solution culture, induced by the introduction of the chloride ion, rather than to direct effects of this element on the metabolism of the plant. There is no convincing evidence that this element is essential for plants, although it is doubtful if any plant has ever been grown in an environment—artificial or otherwise—in which at least traces of chlorine were not present.

Plants indigenous to salt marshes and saline soils can endure the presence of relatively large quantities of chlorides, usually sodium chloride, in the soil. Asparagus is an example of a crop plant which not only tolerates but actually requires treatment with sodium chloride for its best development. Salting of asparagus beds has long been a standard agricultural practice.

Aluminum.—This element is one of the most abundant of those present in the soil, although it occurs chiefly in insoluble forms. A larger proportion of soluble aluminum is generally present in relatively acid soils (below pH 5.0) than in soils of higher pH.

Aluminum is probably universally present in plants although in terms of percentage composition the amount in the ash of most species is very small. While aluminum is not considered to be one of the essential elements it is

known to have a number of pronounced effects upon plants. Except in very dilute concentrations aluminum is distinctly toxic to plants. Its toxicity to such species as corn and barley may become evident in concentrations as low as one part per million in culture solutions. The detrimental effect of soils with a pH of 5 or less upon the growth of some species is undoubtedly due, at least in part, to the toxic effect of the relatively high concentration of aluminum ions in such soils. The beneficial effect upon the growth of some species of adding lime or phosphates to acid soils is at least partly due to a reduction in the solubility of the aluminum compounds present.

Symptoms of Mineral Element Deficiency.—Absence or deficiency of any of the necessary mineral elements (including nitrogen) in the soil or other substratum in which a plant is rooted will sooner or later become apparent in the development of that plant. An insufficient quantity of any of the essential elements in a plant in an available form will result in the production of growth aberrations which are symptomatic of lack of an adequate internal supply of that element. In a general way such deficiency symptoms are common to all species of plants. Certain of these deficiency symptoms assume, however, more or less distinctive aspects in many species. For example, manganese deficiency results in the development of a characteristic mottled chlorosis in the leaves of many species. In maize and other cereals, however, this chlorosis assumes the pattern of an alternate yellow and green striping running lengthwise of the leaves. For this reason it is important that the symptoms of mineral element deficiency be studied for each economic species of plants individually. Once such symptoms have been distinguished for any species they are of assistance in diagnosing abnormal development of plants of that species under natural or cultural conditions.

In Table 41 are summarized the more easily recognized deficiency symptoms for the nine principal essential mineral elements. In such a summary table these symptoms can be described in only a very generalized way, and in many species some deviations from these symptoms are to be expected. The effects of mineral element deficiencies upon the development of the tobacco plant are illustrated in Fig. 96.

Solution and Sand Cultures.—Much of our knowledge regarding the rôle of mineral elements in plants has been obtained by means of solution culture experiments. The growing of plants in solution and sand cultures is today one of the most widely used experimental techniques employed by plant physiologists. The necessary solutions, often rather inappropriately called "nutrient solutions," are prepared by dissolving salts in certain definite proportions in distilled water. A multitude of combinations and concentrations of salts have been suggested for use in solution culture work. In gen-

TABLE 41—SYMPTOMS OF MINERAL ELEMENT DEFICIENCY IN PLANTS

Deficient element	Plant as a whole	Leaves	Stems	Roots	Reproductive organs
Nitrogen	Stunted in growth.	Relatively small, thin, yellowish green or light lemon color. Often abundant anthocyanins in veins.	Slender, woody, light to yellow green in color, often excessive anthocyanins.	Stunted, but usually proportionally less so than the tops.	Fruit chlorotic, develops slowly. Small when mature, often brilliant red (apples, etc.). Seeds light in weight.
Sulfur		Yellowish chlorosis, showing first along veins, at least in some species.	Often relatively slender, sometimes elongate.		
Phosphorus	Slow growing, often dwarfed at maturity.	Dark green color. Anthocyanin development often enhanced. Irregularly distributed brown patches common.	Slender, relatively woody.	Stunted, often proportionately less so than tops.	Fruits slow ripening, relatively small. Seeds late maturing and relatively light in weight.
Calcium	Stunted, stiff, woody. Tendency for symptoms to appear first in younger parts. Plants often die prematurely.	Hard and stiff, often yellowish. Mottling or brown spots common.	Short, stiff and woody, often yellowish. Stem tips die.	Stubby, profusely branched. Meristematic cells die at root tips.	Little or no fruiting.
Potassium	Plants at first stunted, later dry up to a brownish color.	Dull green, sometimes yellow, edges and tips often "scorched." Bronze-colored spots develop. Older leaves affected first.	Usually slender, often with brown streaks.	Usually slender.	Seeds often fail to mature; when they do are relatively small in size.

Iron	Tendency for chlorosis of all aerial parts.	Uniform or at first mottled yellowish or white chlorosis over entire leaf, appearing first in younger leaves. Often necrotic areas in leaves.			
Magnesium	Stunted with differential chlorosis as described in next column.	Mottled chlorosis develops first in *older* leaves. Veins remain green while leaf web tissue turns yellow or whitish.		Usually stunted and sparsely branched.	
Manganese	Differential chlorosis first apparent in growing tips and younger leaves; may later spread to older leaves. Top of plant may thus be yellowish while the lower portions remain green.	Mottled chlorosis—veins green, leaf web tissue yellow or white, first appearing in *younger* leaves. Often necrotic areas in leaves.	Yellowish green, often hard and woody.		
Boron	Growth of plant retarded.	Often burned or spotted.	Apical meristems blacken and die. Stems and petioles often brittle.	Growth checked. Root branches short, stubby and brownish. Root tips often die.	

eral, however, it has been found that most species of plants, under a given set of climatic conditions, grow almost equally well over a considerable range of variations in the mineral salt complex of a solution culture, a sand culture, or a soil.

In 1915 Shive pointed out that the six principal elements—nitrogen, sulfur, phosphorus, calcium, potassium, magnesium—could be supplied by a solution containing only three salts. He proposed a "three salt" solution containing the

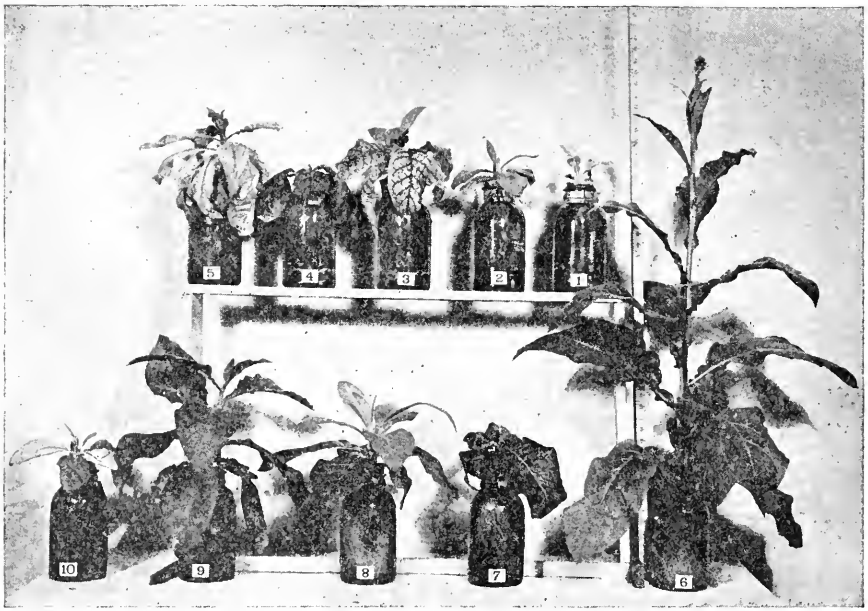

FIG. 96. Symptoms of mineral element deficiencies as shown by tobacco plants. The deficient elements are: (1) nitrogen, (2) phosphorus, (3) potassium, (4) calcium, (5) magnesium, (7) boron, (8) sulfur, (9) manganese, (10) iron. All of the elements were present in (6). Photograph, U. S. Department of Agriculture.

salts $Ca(NO_3)_2$, KH_2PO_4, and $MgSO_4$ to which was added a trace of iron salt. Shive's solution has been a popular one ever since its introduction and these three salts have been used in various proportions and concentrations. However, most present-day investigators use a "four-salt" solution to provide these six elements. Shive and Robbins (1938) suggest a solution made up with the following volume molar concentrations: KH_2PO_4 0.0023 M, $Ca(NO_3)_2 \cdot 4H_2O$ 0.0045 M, $MgSO_4 \cdot 7\ H_2O$ 0.0023 M, and $(NH_4)_2 \cdot SO_4$ 0.0007 M. Minute quantities of iron (as $FeSO_4 \cdot 7H_2O$), boron (as

H_3BO_3), manganese (as $MnSO_4 \cdot 4H_2O$) and zinc (as $ZnSO_4 \cdot 7H_2O$) are also added. A number of species of agricultural plants have been found to give excellent growth when supplied with this solution in either solution or sand cultures.

Hoagland and Snyder (1933) recommend the following stock solution: $Ca(NO_3)_2$ 0.821 g., KNO_3 0.506 g., KH_2PO_4 0.136 g., $MgSO_4$ 0.120 g., water 1 liter, and ferric tartrate 1 cc. of a 0.5 per cent solution repeated at intervals. To this they suggest the addition of an "A-Z" solution which is a supplementary solution containing small quantities of a number of the elements known to be necessary for plants, or which possibly may be necessary. The principal supplementary solution used by these investigators was prepared as follows:

$LiCl$	0.5 g.	$MnCl_2 \cdot 4H_2O$	7.0 g.
$CuSO_4 \cdot 5H_2O$	1.0	$NiSO_4 \cdot 6H_2O$	1.0
$ZnSO_4$	1.0	$Co(NO_3)_2 \cdot 6H_2O$	1.0
H_3BO_3	11.0	TiO_2	1.0
$Al_2(SO_4)_3$	1.0	KI	0.5
$SnCl_2 \cdot 2H_2O$	0.5	KBr	0.5
		Water	18 liters

This solution contains ten elements which have not usually been provided in solution cultures. One cubic centimeter of this solution is added to one liter of stock solution described above.

Such supplementary solutions can also be used with other stock solutions. Most of the species of plants which have been tried have shown better development when the conventional solutions in which they were grown were fortified by the addition of a small quantity of such a supplementary solution than when they were not (Schropp and Scharrer, 1933, and others), although it has by no means been demonstrated that all of the elements present in such solutions are necessary.

In solution culture experiments the prepared solution is transferred to a suitable vessel the size and shape of which depends upon the type of investigation and size of the plants to be studied. Mason jars, crocks of various sizes, and shallow trays are widely used in solution culture investigations. Seedling plants of the species to be studied are then fastened in place in such a way that the root systems are immersed in the solution.

Many precautions must be observed if reliable results are to be obtained with solution cultures. Only a few of the most important of these can be summarized in this discussion: (1) All of the solutions used in any series must have essentially the same osmotic pressure (why?). In general only

solutions with an osmotic pressure of less than 2 atmos. are employed in solution culture work. (2) Either the various solutions used in any one investigation must be buffered to the same pH, or else the influence of differences in their pH must be evaluated. (3) Most species of plants grow better in aerated than unaerated solution cultures. A solution is an abnormal environment for the roots of most species. The oxygen concentration of such aqueous media is relatively low, and carbon dioxide may accumulate as a result of root respiration. Aeration of solution cultures is therefore often necessary and almost always desirable. (4) Provision must be made either for a slow constant renewal of the solution, or else for its frequent replacement with fresh solution if its composition is to be kept even approximately constant. The various ions in a solution are not absorbed at equal rates. Neither is the intake of water proportional to the intake of ions. Furthermore ions, and perhaps certain organic compounds, often diffuse from the roots into the solution. The net result of all these factors is to cause a rapid change in the proportion of elements in any solution culture unless adequate provisions are made to offset these effects. (5) Considerable difficulty is often experienced in keeping the iron in solution cultures in a soluble form. In general the more alkaline the solution the more readily iron passes out of solution. In solutions with a pH of about 6.0 or higher special precautions must be taken to prevent iron deficiency from becoming a limiting factor in plant growth (Hopkins and Wann, 1926 and others). Often the development of iron deficiency in a solution culture can be avoided only by introducing fresh portions of a dilute solution of an iron salt at frequent intervals.

For many types of investigations "sand cultures" are preferred to solution cultures. Suitable vessels are filled with a pure quartz sand which is kept moistened with a nutrient solution. Provision must be made for frequent renewal of the solution, or better, for a continuous supply of solution to the vessel. This is frequently accomplished by means of "drip cultures" in which solution from a reservoir flows into the sand, usually one drop at a time, provision being made for the drainage of excess solution from the vessel (Fig. 97). An important advantage of sand over solution cultures is that in the former the roots grow in a much more nearly normal environment. Maintenance of definite quantitative relations among the various ions is more easily accomplished in solution cultures than in sand cultures, however. Hence for certain types of investigations the former are more useful than the latter.

Employment of the technique of sand and solution cultures has led to many advances in our knowledge of the mineral salt relations of plants. Most of the available information regarding the essentiality of various elements in plant development has resulted from investigations in which a solution or

sand culture method has been used. Much valuable information regarding the specific symptoms of the deficiency of the essential elements in many species of plants has been obtained by this method. By comparing the development of individual plants of a species rooted in a "complete" solution with

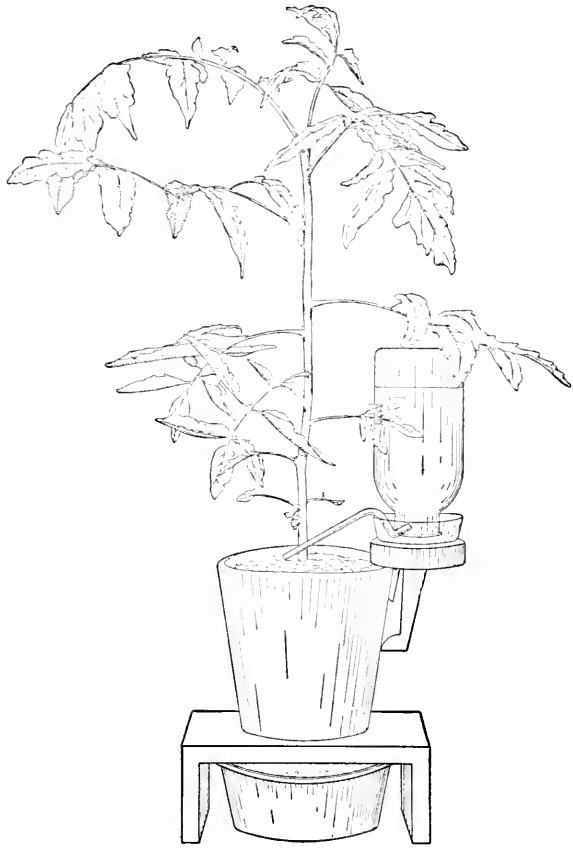

FIG. 97. A "drip culture." Redrawn from Shive and Robbins (1938).

the development of other plants of the same species rooted in a solution lacking in one of the necessary elements, it is possible to determine the distinctive symptoms of a deficiency of that element in plants of that species. Sand and culture solutions are also used in studies of the mechanism of the absorption of ions, and the toxic effects of various ions on plants.

The fact that it has been found possible to grow many species of plants

to maturity in sand or solution cultures has led to attempts to use this as a method for the commercial propagation of crops, a procedure for which the name *hydroponics* has been proposed. Some investigators have used solution cultures (Gericke and Tavernetti, 1936) for this purpose, others sand cultures (Biekart and Connors, 1935) and still others gravel cultures (Withrow and Biebel, 1936). The use of such methods for the commercial production of plants is still in the experimental stage, but it seems probable that the methods of sand and solution cultures can be adapted to the commercial production of at least certain greenhouse crops.

Discussion Questions

1. The addition of inorganic nitrogen to soil in which legumes and grasses are growing together often results in the disappearance of the legumes. What are some possible explanations?
2. The growing of sorghum has frequently been found to be injurious to crops planted later in the same soil. Suggest some possible explanations of this effect.
3. Why can corn plants grow to maturity with their roots in solution cultures while flooding a corn field results in death or serious injury of the plants?
4. Small quantities of copper greatly increase yields of certain crops upon many muck soils. Does this prove that copper is an essential element for these crops?
5. What is a "fertilizer"? What elements are most commonly supplied to plants as fertilizers? List several reasons for the addition of fertilizers to soils.
6. Do you consider it advisable to use the term "plant food" as a synonym for "fertilizer"? Discuss.
7. How does the list of essential elements for plants compare with that for animals?
8. Why are salts of copper and other heavy metals toxic to plants in lower concentrations in solution cultures than in soils?
9. Why does nitrogen deficiency become evident more quickly in plants than sulfur deficiency?
10. List the important ways in which conditions in a solution culture differ from those in a loam soil. Similarly contrast conditions in a sand culture with those in a loam soil.
11. In what parts of a plant would you expect "salt injury" (injury due to over-fertilization) to appear first? Why?
12. Would an overdose of fertilizer be more injurious to plants growing in the bright light or to similar plants growing in the shade? Explain.
13. Can the mineral salt deficiencies of a soil be determined by analyzing the ash of plants growing on that soil?
14. When an increase in yield of a crop is produced by the addition of certain mineral elements to the soil as fertilizers does this prove that those elements were deficient in that soil?

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CHAPTER XXVI

NITROGEN METABOLISM

Proteins and other related nitrogen-containing compounds are the principal constituents of protoplasm and hence are directly or indirectly involved in all of the physiological processes occurring in living cells. Proteins also often occur in plant cells in the form of stored foods, especially in the seeds of many species. Such "reserve" or storage proteins differ in their physical and chemical properties from the protoplasmic proteins. The latter are more complex than the former, they do not respond to the usual protein tests, and they cannot be extracted from tissues by the same methods used in extracting storage proteins. Our knowledge of the synthesis and properties of plant proteins is based almost entirely upon studies of the storage proteins. The structural organization of the protoplasmic proteins is believed, however, to be essentially similar to that of the storage proteins, although in general they seem to be more complex, many of them being of the type known as "conjugate proteins" (see later). In addition to the proteins a number of other kinds of nitrogenous organic compounds occur in plants, some of which play important parts in plant metabolism.

The Proteins.—All proteins contain carbon (50-54 per cent), hydrogen (about 7 per cent), nitrogen (16-18 per cent) and oxygen (20-25 per cent). Although some animal proteins do not contain sulfur this element is apparently present in all plant proteins. The percentage of sulfur in protein molecules never exceeds 2 per cent, however. Phosphorus is also a constituent of certain important types of plant proteins.

The percentage composition of proteins gives no idea of the structure of protein molecules nor of their size. The molecular dimensions of protein molecules are enormous as compared with most other kinds of molecules. Determinations made by the ultracentrifuge method indicate that proteins of one group have molecular weights of 34,500, of another group 68,000, a third 104,000, and a fourth 208,000 and that some have molecular weights as great as 500,000 (Svedberg, 1930). While the molecular weight as determined for a given protein by other methods does not always tally with the value obtained by the ultracentrifuge method, all determinations agree in indicating very high molecular weights for proteins.

Most of our knowledge of the structure of protein molecules has been gained by studying their hydrolytic products. Proteins can be hydrolyzed by treating them with acids, alkalies, or suitable enzymes. The end product of the complete hydrolysis of any protein is always a mixture of amino acids. During the course of protein hydrolysis a number of types of compounds are produced which are intermediate in complexity between the proteins and the amino acids:

Proteins → Proteoses → Peptones → Polypeptides
→ Dipeptides → Amino Acids

It is evident, therefore, that amino acids are the structural units from which the proteins and intermediate products of protein hydrolysis are synthesized in living cells. A consideration of the chemical nature and synthesis of the amino acids is therefore necessary before discussing the proteins further.

The Amino Acids.—Amino acids are, as the name suggests, compounds with the properties of both acids and amines. Every amino acid contains at least one carboxyl (—COOH) group and one or more amino ($—NH_2$) groups. The simplest amino acid is glycine. Glycine may be considered as acetic acid in which one of the hydrogen atoms of the methyl group has been replaced by an amino group:

CH_3COOH — Acetic acid

CH_2COOH
NH_2 — Glycine

In naturally occurring amino acids the amino group, or one amino group if several occur in the molecule, is always attached to the α carbon atom, which is the one next to the —COOH group. Thirty or more different amino acids have been reported by various investigators, but since all of these have not been confirmed, the number of definitely known amino acids is somewhat less. It is also probable that other amino acids remain to be discovered. The names and chemical formulas of the better known amino acids are listed in Table 42.

It should be noted that although proline is a product of protein hydrolysis and is usually classed as an amino acid it contains only an NH group and no NH_2 group.

Absorption of Nitrogen Compounds from the Soil.—No reliable evidence has been obtained that green plants can utilize directly the gaseous nitrogen of the atmosphere in the synthesis of nitrogen-containing organic compounds. Nitrogenous compounds absorbed from the soil serve as the sole source of nitrogen for all terrestrial green plants. Such plants can utilize four

TABLE 42—THE PRINCIPAL AMINO ACIDS

Glycine	$CH_2(NH_2)\cdot COOH$
Alinine	$CH_3\cdot CH(NH_2)\cdot COOH$
Serine	$CH_2OH\cdot CH(NH_2)\cdot COOH$
Threonine	$CH_3\cdot CH(OH)CH(NH_2)\cdot COOH$
Valine	$(CH_3)_2\cdot CH\cdot CH(NH_2)\cdot COOH$
Leucine	$(CH_3)_2\cdot CH\cdot CH_2\cdot CH(NH_2)\cdot COOH$
Isoleucine	$\begin{matrix} CH_3 \\ \diagdown \\ \quad CH\cdot CH(NH_2)\cdot COOH \\ \diagup \\ C_2H_5 \end{matrix}$
Aspartic acid	$HOOC\cdot CH_2\cdot CH(NH_2)\cdot COOH$
Glutamic Acid	$HOOC\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$
Arginine	$HN{=}C(NH_2)\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$
Lysine	$CH_2(NH_2)\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$
Cystine	$\begin{matrix} S\cdot CH_2\cdot CH(NH_2)\cdot COOH \\ \vert \\ S\cdot CH_2\cdot CH(NH_2)\cdot COOH \end{matrix}$
Methionine	$\begin{matrix} \quad S\cdot CH_3 \\ \diagup \\ CH_2 \\ \diagdown \\ \quad CH_2\cdot CH(NH_2)\cdot COOH \end{matrix}$
Phenylalanine	$C_6H_5\cdot CH_2\cdot CH(NH_2)\cdot COOH$
Tyrosine	$HO\cdot C_6H_4\cdot CH_2CH(NH_2)\cdot COOH$
Histidine	$\begin{matrix} HC{=}\!=\!C\cdot CH_2\cdot CH(NH_2)\cdot COOH \\ \vert \qquad\quad \vert \\ N \qquad NH \\ \diagdown \quad \diagup \\ \quad C \\ \quad H \end{matrix}$
Tryptophane	$\begin{matrix} \quad\quad CH \\ \quad\quad \diagup\!\!\diagup \\ HC \qquad C\!-\!\!-\!\!-\!\!-\!C\cdot CH_2\cdot CH(NH_2)\cdot COOH \\ \vert \qquad \vert\vert \qquad\quad \vert\vert \\ HC \qquad C \qquad\quad CH \\ \quad\quad \diagdown\!\!\diagdown \qquad \diagup \\ \quad\quad\quad CH \qquad NH \end{matrix}$
Proline	$\begin{matrix} H_2C\!-\!\!-\!\!-\!\!-\!CH_2 \\ \vert \qquad\quad \vert \\ H_2C \qquad CH\cdot COOH \\ \quad\quad \diagdown \quad \diagup \\ \quad\quad\quad N \\ \quad\quad\quad H \end{matrix}$

kinds of compounds as sources of nitrogen: (1) nitrates, (2) nitrites, (3) ammonium salts, and (4) organic nitrogen compounds. The mechanism of the absorption of ionic forms of nitrogen is believed to be essentially similar to that involved in the intake of other ions (Chap. XXIV). Nitrates apparently can be absorbed by many kinds of plant cells against a concentration gradient.

Most plants absorb most of their nitrogen in the form of nitrates. Normally metabolizing plants usually contain only relatively small quantities of

nitrate, because the nitrogen of nitrate ions is reduced to other forms almost as rapidly as it enters the plant. Under certain conditions, however, plants accumulate relatively large quantities of nitrates in their tissues without any toxic effects. Subsequently such accumulated nitrates may be utilized in the nitrogen metabolism of the plant. Plants sometimes exhibit acute symptoms of nitrogen deficiency while they still contain considerable quantities of nitrates. Although such plants have been able to absorb nitrates, metabolic conditions within the plant have been such that they have been unable to utilize them in the formation of nitrogenous organic compounds.

As shown in the next section the first step in the utilization of nitrates by plants is their reduction to nitrites. It seems probable, therefore, that plants can utilize nitrites as a source of nitrogen, and this supposition has been confirmed by solution culture experiments. However, nitrites are rarely if ever an important source of nitrogen for plants in nature.

Many species of plants when grown in sand or solution cultures under suitable conditions develop as well or better when supplied with ammonium salts as when supplied with nitrates. This is not surprising since the nitrogen in ammonium compounds is in a highly reduced form similar to that found in amino acids and related compounds. In certain types of soils it is probable that ammonium compounds are the chief form in which nitrogen is available to plants. This is apparently true of the acid podsolic soils of northern latitudes and of many uncultivated soils in North Carolina and other southern states. Such soils contain little nitrate, but considerable quantities of ammonium compounds and plants growing in them apparently obtain their nitrogen in the latter form. Unlike nitrate ions plants seldom accumulate appreciable concentrations of ammonium ions.

Even when ammonium fertilizers are applied to agricultural soils much if not most of the absorption of nitrogen by plants occurs in the form of nitrates. In such soils nitrification (see later) usually occurs very effectively, resulting in rapid conversion of ammonium compounds to nitrates.

As a result of the decay of organic remains there are present in most soils at least small quantities of amino acids and other organic nitrogenous compounds. There is considerable evidence that plants can absorb and utilize such compounds in the synthesis of proteins. Under some conditions a considerable proportion of the nitrogen used may be absorbed by plants in the form of such compounds.

Reduction of Nitrates in Plant Tissues.—Since in nitrates the nitrogen is in a highly oxidized state (—NO_3) while in amino acids and other organic compounds it is usually in a highly reduced state, it is evident that reduction of nitrogen is one of the steps in the synthesis of amino acids and other

organic nitrogenous compounds in plants whenever nitrates are the source of nitrogen. Nitrates are first reduced to nitrites and these are further reduced to the NH_2 and NH_3 groups found in organic compounds.

Each of the steps in the reduction of nitrogen requires energy. The observation that nitrates disappear more rapidly from leaves exposed to full sunlight than from leaves in shade or darkness has led to the suggestion that the energy of sunlight can be used directly in the reduction of nitrates. This process can occur in the total absence of light, however, and it is generally agreed that the necessary energy is supplied by the process of respiration. Upon the initiation of nitrate reduction in plant tissues their rate of respiration may increase several fold. Light probably exerts only an indirect effect on this process and the relatively rapid reduction of nitrates in illuminated green tissues is presumably due to the higher carbohydrate content of such tissues. Carbohydrates are not only consumed in respiration during nitrate reduction but are also used in the construction of the organic nitrogenous compounds which are built up during this process.

Eckerson (1924) has succeeded in following microchemically some of the steps in the reduction of nitrates in the tomato plant. Rapidly growing tomato plants were transplanted from good soil to quartz sand when about eight inches tall. The plants were watered with a solution lacking nitrogen until the tissues showed no tests for nitrates, nitrites, ammonia, and amino acids, although they still contained an abundance of carbohydrates. Calcium nitrate was then added to the sand. The nitrate ions were rapidly absorbed and could be detected in all parts of the plant within twenty-four hours. In thirty-six hours *nitrites* were present in considerable amounts at the tips of the stems and in certain other tissues. Traces of ammonia could also be detected. By the end of forty-eight hours the quantity of nitrite had decreased, the amount of ammonium ion in the plants had increased, and a small quantity of asparagine was also found to be present. Three to five days after the addition of the nitrate to the sand around the roots amino acids were present in abundance in the plant tissues and they continued to increase in quantity for three weeks. During the synthesis of these amino acids the carbohydrate content of the cells decreased. The reduction of nitrates within plant tissues is catalyzed by an enzyme known as *reducase*.

Temperature exerts a marked effect on the nitrate reducing capacity of plants. In the tomato plant, for example, although nitrates are almost instantaneously absorbed, their reduction and the synthesis of organic nitrogen compounds occur very slowly at 13° C. At 21° C., on the other hand, both absorption and reduction of nitrate ions occur very rapidly (Nightingale, 1933).

Synthesis of Amino Acids.—Amino acids are synthesized in plant cells from nitrogenous compounds absorbed from the soil and from carbohydrates or their derivatives which have been fabricated in the plant. In the preceding section our attention was focussed on the stages in the reduction of absorbed nitrates. However, on the average about 85 per cent by weight of amino acid molecules is derived from carbohydrates or closely related compounds. It is evident, therefore, that either a shortage of nitrogenous compounds or a deficiency of carbohydrates may retard amino acid synthesis in plant tissues.

Very little is actually known of the chemical mechanism whereby amino acids are synthesized in plants. It is generally considered that ammonia plays a key rôle in this process. This compound usually originates in plant tissues from the reduction of nitrates. Except under unusual metabolic conditions ammonia is present in plant cells only in traces, being apparently utilized in the formation of other compounds as fast as it is produced.

It is also generally believed that certain fatty acids represent an intermediate step between carbohydrates and amino acids in the synthesis of the latter. For example aspartic acid, one of the commonest of plant amino acids, might be synthesized as follows:

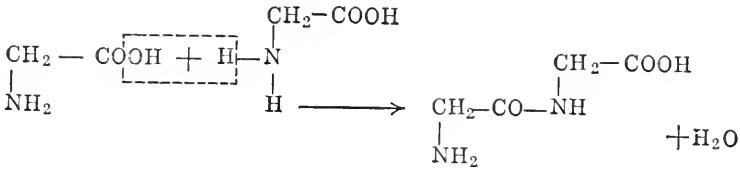
$$\underset{\text{Fumaric acid}}{HOOC\cdot CH{=}CH\cdot COOH} + \underset{\text{Ammonia}}{NH_3} \rightarrow \underset{\text{Aspartic acid}}{HOOC\cdot CH_2\cdot CH(NH_2)\cdot COOH}$$

Other kinds of amino acids may be built up as a result of similar reactions. It is probable that enzymes play a catalytic role in all such syntheses. The synthesis of amino acids in plants is usually accompanied or preceded by the synthesis of asparagine and (or) glutamine (see later).

Cystine is the only amino acid containing sulfur which has been obtained by the hydrolysis of plant proteins. This compound therefore plays an important rôle in the sulfur metabolism of plants. Sulfur always occurs in cystine and other plant sulfur-containing organic compounds in reduced forms. Plants obtain most of their sulfur in the form of sulfates absorbed from the soil, but nothing is known of the chemical mechanism whereby the sulfate ion is reduced in plant tissues.

Amino acid synthesis apparently can occur in most living plant cells. In some species of plants, however, reduction of nitrates and amino acid synthesis takes place principally in the smaller roots, little if any occurring in the aerial organs of the plant. In other species reduction of nitrates and synthesis of amino acids occurs predominantly in the aerial organs (Nightingale, 1937). Examples of plants belonging to the first group are apple, asparagus, and some grasses; examples of species belonging to the second group include peas, soybeans, and tomatoes.

The Synthesis of Proteins.—The theory that proteins are formed by the condensation of numerous amino acid molecules was first suggested by Emil Fischer. He succeeded in linking together eighteen amino acid molecules (fifteen of glycine, three of leucine) and thus producing a synthetic polypeptide in which the amino acids were bound together by peptide linkages. A peptide linkage is one in which the amino group of one amino acid molecule is united with the carboxyl group of another amino acid molecule, water being split off in the process. The simplest dipeptide is that produced by condensation of two molecules of glycine:



The dipeptide formed by the condensation of the two amino acid molecules possesses an amino group and a carboxyl group to which other amino acids can be linked. The addition of amino acid molecules by peptide linkages to either or both of these groups still leaves amino and carboxyl groups present in the resulting molecule. Polypeptides, peptones, proteoses and finally proteins are probably formed by the condensation of more and more amino acid molecules in this way. According to this view a protein molecule is a long chain-like structure composed of hundreds of amino acid residues welded together through peptide linkages.

Some properties of proteins can not be satisfactorily explained in terms of the peptide linkage theory which suggests that other linkages also occur in the natural proteins. Some investigators consider that proteins are condensation products of complex cyclic compounds rather than of amino acids.

Every species of plant or animal produces characteristic and specific proteins which are not found in other species. Therefore a very large number of kinds of proteins must exist. It has generally been considered that the number of possible combinations of amino acids found in proteins is virtually innumerable. Random combination of 19 different kinds of amino acid molecules in the formation of a polypeptide chain of only 50 molecular units could take place in 10^{48} different ways.

Recent investigations by Bergmann and Nieman (1938) seem to indicate, however, that the structure of protein molecules is governed by general principles which greatly limit the number of possible combinations of amino acids. They find that the number of amino acid residues in many simple natural proteins is either 288 or a whole number multiple thereof.

Furthermore, every kind of amino acid in the peptide chain recurs at constant intervals. For example in silk fibroin (a protein) they find that every other amino acid residue is glycine, every fourth one is alanine, and every sixteenth one is tyrosine. This arrangement can be diagrammed as follows, X representing other amino acid residues than those mentioned:

G–A–G–X–G–A–G–T–G–A–G–X–G–A–G–X–G–A–G–X–G–A–G–T–G–A–G–X

A similar recurrence of amino acid residues in a periodic manner was found in the molecules of other proteins. The various kinds of proteins differ from each other in the kinds and frequencies of the constituent amino acids. If the results of these workers regarding the orderliness of the arrangement of amino acid residues in protein molecules is confirmed as a general principle, an important step will have been taken in elucidating the intricate problem of the structure of protein molecules.

The principal regions of protein synthesis in plants do not necessarily correspond to the principal regions of amino acid synthesis. In fact a clear distinction should be made between these two processes. In some species of plants most amino acid synthesis occurs in the roots. In all plants most condensation of amino acids to proteins occurs in meristems or in storage tissues, although some protein synthesis can probably occur in most cells. Amino acids are often translocated from the tissues in which they originate to other, often distant, tissues before being condensed to proteins. It is generally believed that little or no translocation of proteins as such occurs in plants.

In meristems condensation of amino acids results principally in the construction of protoplasmic proteins. This is one phase of the process of assimilation (Chap. XXXI). In many seeds and some other organs condensation of amino acids results in the production of storage proteins. This is a phase of the process of accumulation (Chap. XXXI). Most such proteins are subsequently digested to amino acids which, usually after translocation to other tissues, are assimilated. Reutilization of assimilated proteins may also occur. In senescent leaves, for example, decomposition of protoplasmic proteins may take place and some of the resulting organic nitrogenous compounds, at least, may be translocated to meristems and there resynthesized into proteins. The condensation of amino acids in the formation of proteins is probably catalyzed by enzymes of the proteinase group (Chap. XXVII).

The *nucleoproteins* are a group of complex proteins which constitute a large proportion of the proteins in the nuclei of both plant and animal cells. The chromosomes are apparently composed chiefly of nucleoproteins,

hence these compounds probably play a part in the transmission of hereditary factors from cell to cell and from generation to generation.

Nucleoproteins are formed by combinations between proteins and nucleic acids. Nucleic acids are complex compounds which upon hydrolysis yield phosphoric acid, a carbohydrate (usually *d*-ribose), two purine bases, and two pyrimidine bases. The molecules of both the latter classes of compounds contain nitrogen and are cyclic in structure.

The various steps in protein synthesis are indicated diagrammatically in Fig. 98.

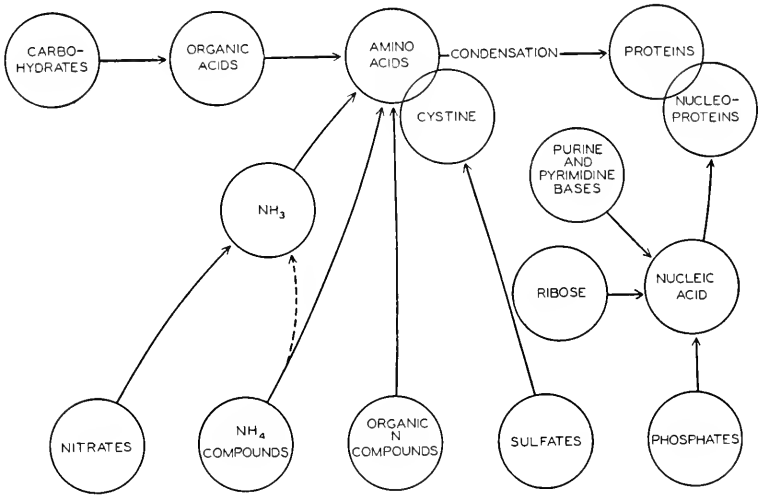


FIG. 98. Diagram illustrating stages in the process of protein synthesis in plants.

The Classification of the Proteins.—The enormous size and complexity of protein molecules together with the fact that their molecular structure is only imperfectly understood has made it impossible to classify them upon a strictly chemical basis. It has been necessary to use certain physical properties such as solubility [1] in acids, alkalies or salt solutions and coagulation or precipitability as a basis for their classification. The "American" system of classification recognizes three main groups of natural proteins:

I. *The Simple Proteins.*—These are proteins which yield only amino acids upon hydrolysis with acids or enzymes. The simple proteins are classified into sub-groups upon the basis of their solubilities:

[1] The great size of protein molecules makes it probable that most protein "solutions" are actually colloidal sols. The term "dispersibility" might describe their behavior more accurately than solubility.

1. *Albumins.* The albumins are water soluble and are coagulated by heat. They occur in both plants and animals and are commonly present in seeds. *Leucosin* from cereals, *legumelin* found in legume seeds, and *ricin* from the castor bean seed are examples.
2. *Globulins.* These proteins are insoluble in water but are soluble in dilute solutions of neutral salts. Globulins are found in many seeds. *Edestin* from hemp seeds and *tuberin* from potato tubers are examples.
3. *Glutelins.* Proteins of this group are insoluble in water and in dilute salt solutions but are readily soluble in dilute acids and alkalies. *Glutenin* in wheat, *oryzenin* in rice and *glutelin* from corn are examples.
4. *Prolamines.* These proteins are insoluble in water, dilute salt solutions, dilute acids and alkalies, and absolute alcohol but are soluble in 70-90 per cent alcohol. *Gliadin* from wheat, *zein* from corn, and *hordein* from barley are examples. Aside from one member of the group which has been extracted from milk all the prolamines have been found in the seeds of cereals.
5. *Albuminoids.* Proteins of this group are insoluble in dilute acids and alkalies, and in solutions of all neutral solvents. *Keratin* from hair and *fibroin* from silk are examples. *Gelatin* is also classified in this group although it does not conform strictly to the definition given. No members of this group are known to occur in plant tissues.
6. *Histones.* These are soluble in water and dilute acids but are insoluble in very dilute ammonia. They are not coagulable by heat. The *globin* of hemoglobin is the most familiar example. No members of this group are known to occur in plant tissues. The known histones do not contain sulfur.
7. *Protamines.* Proteins of this group are soluble in water and ammonia, and form salts with mineral acids. They are not coagulable by heat. Like the two preceding groups the protamines have not been found in plant tissues. These are the simplest known natural proteins in terms of both number and kinds of constituent amino acids. They have been found principally in fish sperm. The known protamines do not contain sulfur.

II. *The Conjugate Proteins.*—The members of the group are proteins with which is combined some non-protein group. They are classified upon the basis of the non-protein group present.

1. *Nucleoproteins* are complex compounds composed of one or more protein molecules combined with a nucleic acid. They are found in cell nuclei.

2. *Glycoproteins* or *Glucoproteins* are proteins combined with a carbohydrate radical. They are not known to occur in plants.
3. *Phosphoproteins* are compounds of proteins and an unknown non-protein complex which contains phosphoric acid in a different linkage from those found in nucleic acids or the phospholipids. The *casein* of milk is a familiar example of this type of protein.
4. *Chromoproteins* are proteins that are combined with a non-protein group which is colored. The hemoglobin of the blood belongs in this group as do the colored proteins present in certain marine algae.
5. *Lecithoproteins* are combinations of proteins with lecithin or some other phospholipid. They occur as constituents of the cytoplasm.

III. *The Derived Proteins.*—This group includes products that are formed as a result of the partial hydrolysis or decomposition of the simple proteins. The proteoses, peptones and peptides are among the better known representatives of the derived proteins. The coagula obtained upon heating many proteins are other examples of this group.

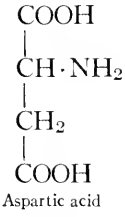
Colloidal Properties of Proteins.—Although some proteins can exist in true solution or in the form of crystals most of them occur in living organisms in the colloidal state. The individual molecules of some proteins are so large as to bring them within the size range of colloidal micelles. The colloidal micelles of other protein sols are probably aggregates of a number of molecules. The significant point, however, is not whether the micelles are composed of one molecule or many, but that they are micelles. In general the properties of protein sols and gels are those of hydrophilic sols and gels in general, as discussed in Chap. V.

Other Compounds Playing an Important Part in the Nitrogen Metabolism of Plants.—1. *Ammonia.*—This compound is supposed to play a central rôle in the nitrogen metabolism of plants, although ordinarily it does not occur in plant cells in more than traces. There are three possible sources of ammonia in plants: (1) Reduction of nitrates produces ammonia, (2) Ammonia may be absorbed directly from the soil, principally in the form of ammonium ions, (3) Ammonia is also sometimes set free in plant cells upon the oxidation of amino acids or related compounds. As described later ammonia released in this way is usually tied up in the synthesis of asparagine, glutamine, and urea, although if the cells are markedly deficient in carbohydrates ammonia may accumulate in them.

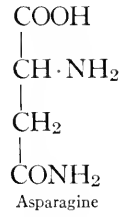
2. *Ammonium Salts.*—Ammonium salts do not commonly occur in plant cells in significant concentrations. However, in species with a distinctly acid

cell sap such as begonia and rhubarb, ammonia often reacts with organic acids forming salts (Ruhland and Wetzel, 1926, 1927).

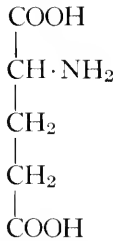
3. *Asparagine and Glutamine.*—The compound resulting if one of the carboxyl groups of aspartic acid is replaced with an acid amide group is called *asparagine*. A similar substitution in the molecule of glutamic acid gives the compound *glutamine*, thus:



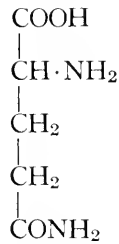
Aspartic acid



Asparagine



Glutamic acid



Glutamine

One or both of these compounds are apparently found in all species of plants. The rôle of asparagine has been more exhaustively studied than that of glutamine (Robinson, 1929, Murneek, 1935). Asparagine appears in relatively large quantities in germinating seeds, being especially abundant in those of legumes. The asparagine thus produced is apparently synthesized from amino acids resulting from the digestion of proteins. Asparagine is formed in plants only in the presence of oxygen. Protein hydrolysis will occur in the absence of oxygen and under such conditions amino acids are produced but no asparagine.

Asparagine is a very mobile compound and is an important translocation form of nitrogen. Much of the asparagine synthesized in seeds is translocated to growing regions of the seedlings and there utilized in the synthesis of proteins. Similarly when proteins are hydrolyzed in other organs of a plant asparagine usually appears as a product along with amino acids. Asparagine also serves as a temporary storage form of nitrogen in some plant organs.

When plants are deficient in carbohydrates, amino acids produced as a result of protein digestion may be oxidized in respiration (Chap. XXIX).

Under such conditions asparagine and other amides are usually synthesized. The amino groups set free, probably as ammonia, in the oxidation of amino acids are probably used in the construction of asparagine or other amide molecules. If carbohydrate deficiency becomes severe asparagine may also be oxidized, resulting in the liberation of ammonia in the plant cells (Mothes, 1926). Under such conditions the concentration of ammonia sometimes becomes great enough to exert a toxic or even lethal effect on the cells since free ammonia is a cell poison. Ammonia toxicity during carbohydrate starvation is apparently obviated except in extreme cases by the synthesis of asparagine. In general accumulation of ammonia in a plant from any cause seems to favor synthesis of asparagine (Prjanischnikow, 1924).

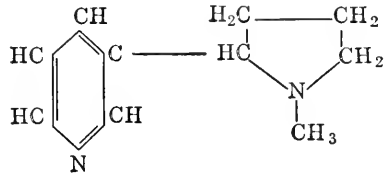
Glutamine is believed to play a rôle in plant metabolism essentially similar to that of asparagine. In some plants the quantity of glutamine exceeds that of asparagine, although the converse is apparently more often true. Etiolated castor bean seedlings, for example, may contain four times as much glutamine as asparagine. There is also some evidence that asparagine and glutamine residues occur in protein molecules.

4. *Urea.*—Urea ($(NH_2)_2 \cdot CO$) occurs abundantly (as much as 11 per cent of the dry weight) in some fungi and is also present in small quantities in certain seed plants (Klein, *et al.*, 1930). Urea is one of the products formed when the amino acid arginine is hydrolyzed by the enzyme *arginase* (Chap. XXVII). Urea, like asparagine and glutamine, may also be one of the products of the oxidation of amino acids in tissues with a low carbohydrate content. Under the influence of the enzyme *urease* urea is hydrolyzed to ammonia and carbon dioxide (Chap. XXVII). Some investigators believe that urea may serve as the starting point in the synthesis of some of the complex cyclic nitrogen compounds which appear to be present in some proteins but there is no actual evidence in favor of this view. The physiological rôle of urea in the seed plants is probably very similar to that of asparagine and glutamine.

5. *The Alkaloids.*—Alkaloids are complex cyclic compounds containing nitrogen that are produced only in certain species of plants. They are especially common in members of the *Solanaceae, Papaveraceae, Leguminosae, Ranunculaceae, Rubiaceae*, and *Apocynaceae*. Species of plants which contain one alkaloid are very likely to contain others. More than twenty different alkaloids have been isolated from opium, which is the dried juice of the unripe fruits of certain species of poppies.

Some of the better known alkaloids are *nicotine*, from tobacco, *quinine* from the bark of the cinchona tree, *morphine* from poppy fruits, *strychnine* and *brucine* from the seeds of *Strychnos nuxvomica*, and *atropine* from the

deadly nightshade (*Atropa belladonna*). *Caffeine* from coffee, tea, etc. and *theobromine* from the cocoa bean are often also classed with the alkaloids, although actually they are purine derivatives. The formula for nicotine, a representative alkaloid, gives some idea of the chemical nature of these substances:



Most of the alkaloids are white solids, but nicotine is a liquid at ordinary temperatures. They are all basic in reaction as the name indicates, and only slightly soluble in water. The alkaloids are usually found in plant tissues in the form of salts of organic acids.

The physiological rôle of the alkaloids in plants, if any, is unknown. Their restricted distribution suggests that they are not involved in any processes of general importance. They are probably by-products of the nitrogen metabolism of the species in which they are found. Most of them have pronounced physiological effects on the human body, and a number are of therapeutic importance.

Nitrogen Metabolism in Relation to Carbohydrate Metabolism.—Both carbohydrate and proteinaceous foods are necessary for the development of any plant. Deficiency of either soon results in the development of characteristic and recognizable growth abnormalities. The supply of carbohydrate and proteinaceous foods in a plant is influenced by many factors. Among these are reciprocal relationships between available carbohydrates and available organic nitrogenous compounds. As already described, synthesis of amino acids, etc. occurs only at the expense of carbohydrates or their derivatives which serve both as building material (together with nitrates) and as a source of energy. Rapid amino acid synthesis therefore usually results in a diminution in the proportion of available carbohydrates in a plant, while plants in which amino acid synthesis occurs slowly will usually be proportionately rich in carbohydrates.

Kraus and Kraybill (1918) and subsequently many other investigators have sought to give expression to the metabolic rôles of carbohydrates and organic nitrogenous compounds in terms of the relative proportions of the two in the plant (often called the "carbohydrate-nitrogen ratio"). Working with tomato plants they recognized four different metabolic conditions in terms of the proportionate amounts of these two types of substances present.

Each of these conditions was distinguished by characteristic morphological responses on the part of the plants:

I. Very low proportion of available carbohydrates to available nitrogen. Plants were weakly vegetative and unfruitful. The few flowers which formed abscised. Stems were slender, succulent and a light grayish-green in color. Chemical analysis showed that the plants contained practically no reserve carbohydrates, but a relatively large quantity of available nitrogenous compounds.

II. Low proportion of available carbohydrates to available nitrogen. Plants were vigorously vegetative but unfruitful. Stems were thick, pithy, and succulent. Leaves were large, soft, and dark green. Chemical analysis showed the plants to be low in reserve carbohydrates, but relatively high in available nitrogenous compounds.

III. Intermediate proportion of available carbohydrates to available nitrogen. These plants had proportionately more carbohydrates than those in class II but nevertheless were not deficient in nitrogen. They exhibited good vegetative growth and good reproductive development. Stems were thick and relatively woody. Leaves were well developed and a normal green in color. Chemical analysis showed these plants to contain considerable reserve carbohydrate and to be lower in available nitrogenous compounds than those of class II.

IV. High proportion of available carbohydrates to available nitrogen. Plants were feebly vegetative and unfruitful. Most flowers abscised, although a few small, woody fruits were set. Stems were slender, hard, and woody. Leaves were small, stiff, and light green to yellow in color. Chemical analysis showed the plants to contain relatively large quantities of stored carbohydrates, but very little available nitrogen in any form.

Any interpretation of the results of Kraus and Kraybill and the similar results of a number of other investigators must be based on an evaluation of the relative rôles of carbohydrate and nitrogenous foods at different stages in the growth cycle of a plant. The best "carbohydrate-nitrogen ratio" for one stage in the development of a plant is not necessarily the best for other stages. For example, in the wheat plant the proportion of available carbohydrates to available nitrogenous compounds increases progressively throughout the vegetative period, and flowering occurs when this proportion becomes sufficiently high (Hicks, 1928).

The effects of carbohydrate and nitrogen metabolism on vegetative development will first be considered. Next to water proteins are the chief constituent of the protoplasm of active cells, while the cell walls are constructed almost entirely of carbohydrates. Continued development of new

cells by any meristem therefore requires a supply of both carbohydrate and proteinaceous foods (largely amino acids). Considerable quantities of carbohydrates are also consumed in any actively growing meristem in the process of respiration.

With the sole exception of water it is these two types of compounds which are used in the greatest quantities by meristems in the construction of new cells. If the supply of amino acids, etc. to any growing meristem is abundant relative to the supply of carbohydrates a large quantity of protoplasm will be synthesized relative to the amount of cell wall material formed. The resulting cells will usually be large, thin-walled, and well stocked with protoplasm. Tissues composed largely or entirely of such cells are usually soft and succulent and contain very little mechanical tissue.

If the converse situation prevails, and carbohydrate foods are relatively more abundant than organic nitrogenous compounds, proportionately more cell wall material and less protoplasm will be synthesized. The resulting cells will be small, thick-walled, and contain only relatively small quantities of protoplasm. Tissues composed largely or entirely of such cells are usually compact and more or less woody. The development of fibers or other mechanical tissues is favored by nitrogen deficiency, as is also the formation of cutin on leaves.

Since both types of compounds are required for normal vegetative growth, extreme deficiency of either organic nitrogenous compounds or of carbohydrates results in a stunting of plants. Dearth of the former limits growth because little or no new protoplasm can be synthesized; dearth of latter largely or entirely checks synthesis of new cell wall material and also checks formation of protoplasm because carbohydrates are required in amino acid synthesis. The plants in class IV of Kraus and Kraybill's classification are clearly *nitrogen deficient* plants (*cf.* Table 41). Correspondingly those of class I are obviously *carbohydrate deficient* plants.

The type of vegetative development exhibited by the plants of class II is characteristic of those receiving an excess of nitrogen, but which nevertheless contain a sufficient supply of carbohydrates to permit the maintenance of vegetative growth. In such plants a large proportion of the carbohydrates synthesized are converted into amino acids which favors the development of large, thin-walled cells, and a generally rank or succulent growth habit. The vegetative responses of the plants in class III are characteristic of those in which the proportion of carbohydrates to organic nitrogenous compounds is somewhat greater than in class II. Cells in general are smaller, their walls are thicker, and the tissues in general are relatively firm although not excessively woody.

The effect of the relative availability of carbohydrate and organic nitrogenous compounds upon the reproductive phase of growth is a more complex phenomenon than the influence of the proportionate quantities of those two foodstuffs upon vegetative development. The reproductive process involves a complex series of phenomena among the more important of which are the initiation and maturation of essential floral parts, the development of megaspores and microspores, the formation of viable egg cells and pollen, pollination and growth of the pollen tube, fertilization, and finally development of the embryo, seed, and fruit. Different stages in this process are variously influenced by the carbohydrate and nitrogen metabolism of plants.

In perennial species further complications arise from the fact that differentiation of floral parts may occur during one growing season, but their maturation does not take place until the next one. Certain phases of the development of perennial species may be influenced as much by the carbohydrate-nitrogen metabolism of the preceding season as by that of the current year.

In many annual species the number of flowers produced is conditioned partly by the extent of the previously developed vegetative body of the plant. Hence if metabolic conditions do not favor vegetative development during the earlier stages of the growth cycle this may result in a decrease in the number of flowers produced.

Although flower primordia apparently will form on plants over a wide range of metabolic conditions, in general occurrence of the complete cycle of reproductive processes may be checked by a lack of either carbohydrate or nitrogenous foods. A deficiency of carbohydrates has been found to induce microspore degeneration and pollen sterility in the tomato (Howlett, 1936) and apparently has a similar effect in many other species. Nitrogen deficiency, on the other hand, seems to have relatively little effect on anther development and pollen fertility. The converse appears to be true of the female organs. Their development is not greatly influenced by carbohydrate deficiency, but is markedly repressed by nitrogen deficiency. In hemp (a dioecious species) the male plants characteristically have a higher carbohydrate content than the female plants, but the nitrogen content of the latter is higher (Talley, 1934).

The development of fruits is often physiologically the equivalent of the initiation of a new but morphologically different cycle of vegetative growth. This is especially true of fruits of the succulent, juicy type such as tomato. The formation of the cells characteristic of such organs is usually favored by a relatively high proportion of nitrogenous foods. The proportionate amount of carbohydrate and proteinaceous foods which favors the best development of fruits undoubtedly varies greatly, however, with different species.

The Origin of Nitrogenous Compounds in the Soil.—The entire nitrogen supply of the higher plants is obtained from compounds in the soil that contain this element. Nitrogen compounds are continually being lost from the soil by the leaching action of rains and by the removal of the plant cover through fire or other agencies. Large quantities of nitrogen are lost every year from cultivated soils when crops are harvested. The soils, however, show no equivalent depletion of their nitrogen supply. Since there is no nitrogen in the rocks from which the soils are derived it is obvious that the supply of nitrogenous compounds in the soil must constantly be replenished in some way. Maintenance of a supply of nitrogenous compounds in the soil is accomplished principally by the activities of certain soil organisms, the *nitrogen fixing* bacteria. The process of *nitrogen fixation* and other important phenomena influencing the soil nitrogen supply will be briefly discussed in the following paragraphs.

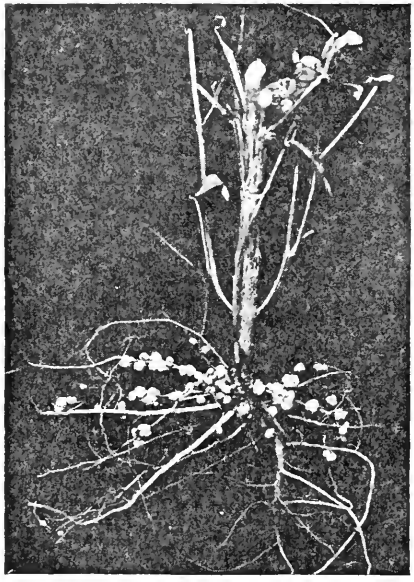


FIG. 99. Nodules containing nitrogen-fixing bacteria on roots of soy bean. Photograph courtesy of Dr. H. W. Batchelor.

1. *Nitrogen Fixation.*—Two groups of bacteria are able to fix appreciable quantities of atmospheric nitrogen in organic combination: (1) certain saprophytic bacteria which obtain their energy from dead organic matter in the soil and (2) symbiotic nitrogen fixing bacteria which live in the roots of members of the legume family. Other bacteria and some fungi may also be able to combine atmospheric nitrogen with organic compounds but the amount of the nitrogen fixed by these organisms is probably not important.

Symbiotic nitrogen fixation is the result of the activities of species of *Rhizobium* which are rod-shaped bacteria that enter the roots of legumes by way of the root hairs and cause the formation of nodules on the young roots (Fig. 99). These bacteria live inside the nodules and there synthesize organic nitrogen compounds from the carbohydrates of the host and gaseous nitrogen of the air.

Some of the nitrogenous compounds synthesized by these organisms are utilized by the legume plants in their protein metabolism, some may move

out of the nodules into the surrounding soil and some remain bound up in the proteins of the bacterial cells themselves. The amount of nitrogen fixation is influenced by a number of factors among the most important of which are the quantity of available nitrogen and carbon compounds present in the soil. When available nitrogen is high nitrogen fixation is retarded. Apart from the depressing effects of high soil nitrogen content, nitrogen fixation by the symbiotic bacteria is, in general, favored by the same factors that promote good vegetative growth of the host plants. Surprising quantities of atmospheric nitrogen may be fixed into organic compounds by these bacteria. Under very favorable conditions a good crop of alfalfa may add as much as 400 pounds of nitrogen per acre to the soil. The average, however, is much lower being estimated by Giobel (1926) as ranging between 100 and 200 pounds per acre when the common legumes are used as host plants.

Most of the nitrogen fixation by saprophytic bacteria is brought about by two groups of organisms: (1) The *Azotobacter* group composed of coccus-like aerobic organisms, and (2) the *Clostridium* group which are rod-shaped anaerobic bacteria. Both types are common in well aerated soils. The aerobic forms occur around the surface of the soil particles while the anaerobic forms are found within aggregations of soil particles or in regions of the soil in which the oxygen content has been depleted by respiration. These bacteria combine the gaseous nitrogen of the air with carbohydrate compounds obtained from the soil. *Azotobacter* is usually absent from soils more acid than pH 6 but *Clostridium* can tolerate soil acidities as great as pH 5. Both groups can operate effectively in relatively dry soils. The quantity of atmospheric nitrogen fixed by the saprophytic bacteria is much less than that combined by the symbiotic organisms and it is probable that these organisms do not add appreciable quantities of combined nitrogen to the soil.

2. *Ammonification.*—In the process of decay the complex nitrogenous compounds present in dead plant and animal tissues are broken down into a number of simpler compounds, most of the nitrogen being released in the form of ammonia. This process is termed *ammonification* and the bacteria involved are called ammonifying bacteria. Ammonification is not the result of the activities of a single group of bacteria but may be brought about by a large number of different micro-organisms, including the actinomyces and filamentous fungi in addition to numerous groups of bacteria. Probably most of the bacteria commonly present in soils are concerned in the formation of ammonia from one kind of nitrogenous material or another. The amount of ammonia produced in the decay of nitrogenous materials is influenced by (1) the available carbohydrate supply, (2) the chemical composition of the nitrog-

enous materials, (3) the organisms concerned, and (4) the acidity, aeration, and moisture content of the soil. The ammonifying organisms utilize carbohydrates as a source of energy more readily than proteins so the amount of ammonia produced is markedly decreased when large amounts of carbohydrates are available.

3. *Nitrification.*—The ammonia produced in the decomposition of proteins and other organic nitrogenous compounds may be acted upon by the so-called nitrifying bacteria and transformed in two steps to nitrates. The first step is the oxidation of ammonia to nitrites. This is accomplished by organisms belonging to the two genera: *Nitrosomonas* and *Nitrosococcus*. Neither of these organisms can oxidize the nitrite which they produce but this compound is oxidized to nitrates by a different organism, *Nitrobacter*. All of these organisms differ from each other morphologically but they are similar physiologically in that they use the energy obtained from the oxidation of ammonia or nitrites in the synthesis of carbohydrate compounds from carbon dioxide and water. The carbohydrates synthesized apparently are not used as a source of energy but are utilized only in assimilation (Chap. XXXI). The respiration of the nitrifying bacteria is therefore essentially different from that of the higher plants and from most other non-green plants in that they obtain their energy by the oxidation of ammonia or nitrites rather than from carbohydrates. The soil conditions favoring nitrification are: (1) pH values on the alkaline side of neutrality, (2) the absence of large amounts of carbohydrates in the soil and (3) good aeration.

4. *Denitrification.*—A large number of organisms are capable of reducing nitrates to nitrites and ammonia. This occurs commonly in the tissues of the higher plants as we have seen earlier. Certain soil organisms, however, can attack nitrates and reduce them to molecular nitrogen. These organisms are known as denitrifying bacteria and they include a number of species of which *Bacterium denitrificans* is probably the best known. Denitrification occurs only in the absence of atmospheric oxygen and most effectively when an abundant supply of carbohydrates is present in the soil. It does not normally occur in well cultivated soils.

Small amounts of inorganic nitrogen compounds reach the soil from the atmosphere. Oxides are formed during electrical storms and these are brought into the soil by the rain. Ammonia also escapes from various sources into the atmosphere and may be returned to the soil in solution in raindrops. Measurements made at the Rothamsted Experiment Station in England over a five year period showed that rain water brought down 4.4 pounds of nitrogen per acre per year. The amount of nitrogen added to the soils at Rothamsted in this way approximately equalled losses of nitrogen suffered by leaching.

The complete story of nitrogen in relation to plant life involves a whole series of events, some of which occur in the cells of micro-organisms of the soil and some in the tissues of the higher plants. This series of events is frequently referred to as the "nitrogen cycle." The interrelations of the various processes concerned in the nitrogen cycle are summarized diagrammatically in Fig. 100.

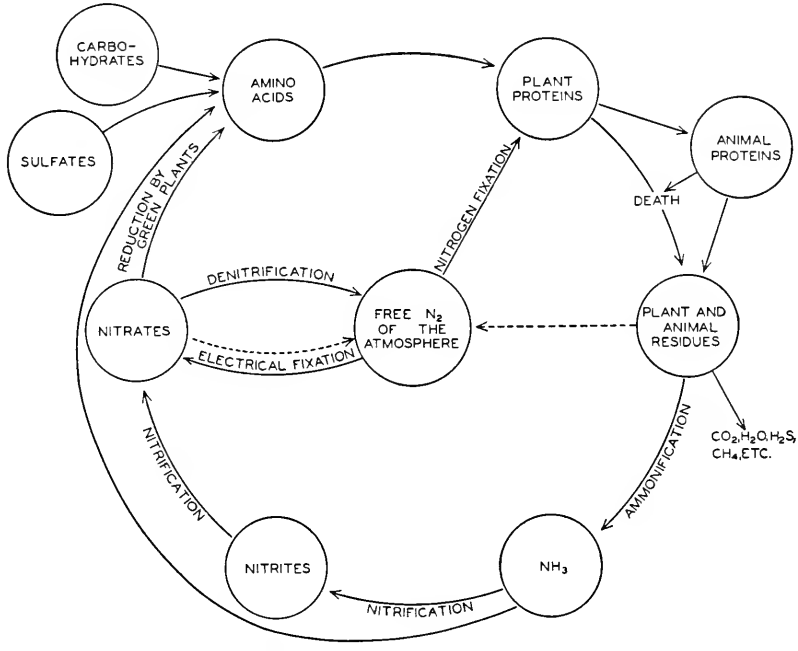


FIG. 100. The nitrogen cycle.

Mycorrhizas.—The roots of many species of plants are regularly infected with the mycelium of fungi. Such a root, together with its associated fungal hyphae, is called a *mycorrhiza* (literally "fungous-root"). In the *ectrotropic* mycorrhizas the mycelium is chiefly external to the root, investing it with a web-like mantle of hyphae. Some hyphae also penetrate into the root, infecting principally the cortex. In the *endotropic* mycorrhizas the hyphae are intracellular, being found principally within the cells of the epidermis and the cortex. Roots which become infected with mycorrhizal fungi stop elongating and often branch extensively. Mycorrhizas are therefore usually short and stubby as compared with uninfected roots on the same plant. Many authorities believe that mycorrhizas are present on the roots of the majority of vascular species.

Ectrotropic mycorrhizas are found on many forest tree species such as beeches, oaks, hickories and many conifers. They are particularly abundant on trees growing in soils rich in humus. The fungal associates in forest tree mycorrhizas are mostly members of the group of *Basidiomycetes*, many of them apparently being common woodland species of mushrooms.

Endotropic mycorrhizas are found on many species of the orchid, heath, and gentian families, and also on some trees, such as the red maple and walnut. The fungous associates in such mycorrhizas are apparently mostly microscopic molds.

Diverse opinions have been advanced regarding the significance of these root-fungus associations. Undoubtedly the mycorrhizal fungi associated with chlorophyllous plants obtain most or all of their carbon-containing compounds from the species on which they grow. Hence some authorities consider them to be no more than root parasites. At least some mycorrhizas, however, are apparently beneficial to their associates. This seems to be especially true of the mycorrhizas of conifers, particularly when they are growing in distinctly acid soils which are low in nitrates. There is evidence that on such soils mycorrhizas aid in making available to their host plants some of the nitrogen of the complex organic compounds found in the humus (Rayner, 1926, 1927).

Discussion Questions

1. List the different kinds of chemical combination in which nitrogen is present in green plants.
2. List and discuss the various ways in which the "C/N ratio" of plants can be increased; ways in which it can be decreased.
3. Why does not the continuous occupancy of an uncultivated area by native plants eventually exhaust all the nitrogen in the soil?
4. Corn (maize) was planted upon a field that was in alfalfa the preceding year. Trace the origin of the various kinds of nitrogen compounds which would be available to the corn plants.
5. What are some of the important inter-relationships between respiration and the nitrogen metabolism of plants?
6. How could you demonstrate convincingly that green plants cannot use the nitrogen of the atmosphere?
7. It is often possible to induce the resumption of flowering and fruit production in old apple trees that have ceased bearing by heavy pruning coupled with the application of nitrogenous fertilizers to the soil. Explain.
8. In general would you expect soil nitrogen conditions which are favorable to the development of good crops of lettuce or celery to be equally favorable for the development of Irish potatoes? Explain.
9. In general would you expect the "C/N ratio" of a tomato plant to be higher if grown at 15° C. or if grown at 25° C. Explain.
10. Mature fruit trees often flower unusually heavily the spring following a dry year. Explain.

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CHAPTER XXVII

DIGESTION

Digestion.—The conversion of complex, usually insoluble foods into simple, usually soluble forms, in or through the agency of living organisms is known as *digestion.* The most familiar digestive reaction in plants is the conversion of starch to glucose. The summary equation for this process is:

$$\underset{\text{Starch}}{(C_6H_{10}O_5)_n} + n\,H_2O \rightarrow n\,\underset{\text{Glucose}}{C_6H_{12}O_6}$$

Actually, as already discussed in Chap. XXII, this reaction takes place in several stages.

Digestion of starch is a process of hydrolytic decomposition. This is true of all other digestive processes, as will be evident from other equations given later in the chapter. Furthermore the reaction representing the digestion of any substance is an exact reverse of the condensation reaction by which it was originally produced. If the direction of the arrow is reversed in the above equation, for example, it will then represent the process of starch synthesis.

Digestion may occur in any living plant cell, but obviously the quantities of food hydrolyzed are likely to be greatest in those cells in which foods have accumulated in abundance. The leaf cells in which starch manufacture occurs are also a seat of much digestive activity as practically all of the starch accumulated in such cells is sooner or later digested into glucose and translocated in the form of sugars to other tissues of the plant.

The basic chemical mechanism of digestion is, as in a number of other fundamental physiological processes, similar in plants and in animals, although in its mechanical aspects digestive processes in the higher animals are much more complex than in plants. This is due to the presence in all of the more complex forms of animal life of a more or less highly organized system of digestive organs. In the higher plants digestion is usually an intracellular phenomenon. In all living organisms, however, the same fundamental types of foodstuffs—carbohydrates, fats, and proteins—are hydrolyzed in the process of digestion.

Enzymes.—If a sterile suspension of starch and water is protected from contamination by micro-organisms, it may be allowed to stand indefinitely without conversion of any detectable amount of the starch into sugar. In order to accomplish such a hydrolysis in the laboratory it is necessary to treat the starch suspension with a strong acid, and to subject it to a relatively high temperature. In living organisms, however, this starch to sugar transformation, and other similar hydrolytic reactions, occur in media which are seldom strongly acid, and at temperatures which are usually not greatly different from those of the environment. This is made possible by the presence in living organisms of substances known as *enzymes* which partake of the nature of catalysts. In fact enzymes are frequently spoken of as "organic catalysts." Catalysts are substances which accelerate or retard the rate of a reaction, yet are themselves found to be chemically unchanged at the end of that reaction. In most of the known examples of catalysis, including enzymatic reactions, the effect of the catalyst is to accelerate rather than to retard the reaction. It has often been considered that catalysts cannot start a reaction but that they merely influence the speed of a reaction which is already in progress, although perhaps only at an infinitesimal rate. Many contemporary authorities, however, incline to the view that catalysts, including enzymes, can actually initiate reactions.

Among the well known inorganic catalysts are such substances as platinum black, metallic oxides, and various metals in the colloidal state. Many catalysts appear to owe their properties largely to the possession of an enormous specific surface at which free secondary valences are present. These cause the interacting compounds to be adsorbed on the surface of the catalysts. In this adsorption process compounds are brought into such intimate contact on the surface of the adsorbent that reactions are initiated which would not otherwise occur, or at least would proceed at a much slower rate. The compounds produced by such reactions are not, in accordance with the principles of adsorption equilibria, retained in quantity at the surface of the adsorbent. Hence the products of the reactions are released from the surface of the catalyst almost as rapidly as they are formed. The catalytic action of enzymes is also believed to depend on an adsorption mechanism.

Enzymes, as far as is known, are produced only by the protoplasm of living cells. It is not know whether there are definite centers of enzyme synthesis in the protoplasm, or whether this property is possessed by protoplasm generally.

Cells which lack a specific enzyme in its active form are frequently found to contain a chemical precursor of the enzyme which under certain conditions is converted into the enzyme. Such substances are termed *zymogens.*

For example, the enzyme lipase cannot be detected in dormant castor bean seeds, but as soon as they begin to germinate its presence can be readily demonstrated. It is therefore believed that the enzyme is present in the ungerminated seeds as a zymogen and that, upon germination, it is rapidly converted into the enzyme proper. Zymogens may be either intermediate stages in the synthesis of enzymes, or may be compounds in which the enzyme is combined with some component which results in its inactivation.

It is customary to distinguish between *intracellular* or *endoenzymes* and *extracellular* or *exoenzymes*. Endoenzymes are those which operate within the cells in which they are produced, while the exoenzymes are secreted from the cells and accomplish their effects in some outside medium. In the higher animals enzymes are secreted from specialized cells or glands into the various organs of the alimentary tract where they effect the digestive processes. "Ptyalin" (an amylase), the starch hydrolyzing enzyme secreted into the saliva, and pepsin, a protein splitting enzyme secreted into the stomach, are familiar examples of exoenzymes produced in the bodies of the higher animals. Most of the enzymes in the higher plants are endoenzymes while extracellular digestion is a regular feature of the metabolism of many bacteria and fungi. The secretion of exoenzymes by many such species can be readily demonstrated. When, for example, colonies of certain species of bacteria are grown on culture media containing starch, gradual digestion of the starch in a concentric zone around each colony occurs.

The Classification of Enzymes.—Numerous investigations have revealed the presence of a large number of enzymes in living organisms and probably many more remain to be discovered. Since, with a few exceptions, nothing is known of the chemical nature of enzymes themselves, their presence is recognized by the chemical transformations which they catalyze, and such chemical changes provide a basis for their classification. The compound which undergoes chemical transformations under the influence of an enzyme is called the *substrate;* the results of the reaction the *end products.*

The outline classification of the hydrolytic enzymes presented in Table 43 includes all of the better known enzymes in this group. Certain of the so-called enzymes listed in this table are definitely known to be mixtures of two or more enzymes and it is probable that this is also true for some of the others.

With rare exceptions enzymes are named for the substrates upon which they act, the name of the enzyme being derived by appending the suffix *ase* to the name of the substrate, *e.g.* maltose, maltase; cellulose, cellulase; etc.

A classification of the other important group of enzymes found in plants, the oxidizing-reducing enzymes, will be found in Chap. XXX.

TABLE 43—CLASSIFICATION OF THE HYDROLYTIC ENZYMES

Name of enzyme	*Substrate*	*End Products*
I. Carbohydrases		
1. Sucrase	A. Sucrose	Fructose and glucose
	B. Raffinose	Fructose and melibiose
	C. Gentianose	Fructose and gentiobiose
2. α-Glycosidases		
A. Maltase	Maltose	Glucose
B. Trehalase	Trehalose	Glucose
3. β-Glycosidases		
A. Emulsin	All β-glycosides	Sugar and non-sugar(s)
B. Cellobiase	Cellobiose	Glucose
C. Gentiobiase	Gentiobiose	Glucose
4. β-Galactosidases		
A. Lactase	Lactose	Galactose and glucose
B. Melibiase	Melibiose	Galactose and glucose
5. Amylase	Starch, dextrins	Maltose
6. Cellulase	Cellulose	Cellobiose
7. Hemicellulases	Hemicelluloses	Hexoses and pentoses
8. Lichenase	Lichenin	Cellobiose
9. Inulase	Inulin	Fructose
10. Protopectinase	Protopectin	Pectin
11. Pectinase	Pectin, pectic acid	Galactose, arabinose, and galacturonic acid
II. Esterases		
1. Lipases	Fats	Glycerol and fatty acids
2. Chlorophyllase	Chlorophyll *a*	Chlorophyllide *a* and phytol
3. Pectase	Pectin	Pectic acid and methyl alcohol
III. Enzymes Hydrolyzing Nitrogen Compounds		
1. Proteinases		
A. Pepsinases	Proteins	Amino acids or intermediate products of protein hydrolysis
B. Papainases	Proteins	
C. Tryptases	Proteins	
2. Peptidases	Polypeptides	Amino acids
3. Desamidases		
A. Urease	Urea	CO_2 and NH_3
B. Asparaginase	Asparagine	Aspartic acid and NH_3
C. Arginase	Arginine	Urea and ornithine

General Properties of Enzymes.—1. *Catalytic Properties.*—The behavior of enzymes and inorganic catalysts is very similar although enzymes also possess certain properties not common to all catalysts. Reactions catalyzed by enzymes as a rule are more complex than those which proceed under the influence of inorganic catalyzers. As with other catalysts, essentially the

same quantity of enzyme seems to be present at the end of the reaction as at the beginning. A very small quantity of enzyme can catalyze the transformation of relatively large quantities of the substrate. It has been estimated, for example, that the enzyme sucrase ("invertase") can effect the hydrolysis of at least 1,000,000 times its own weight of sucrose without exhibiting any appreciable diminution in its activity. The cessation of enzymatic reactions is usually due, not to a diminution in the effectiveness of the enzyme, but to other causes. All hydrolytic reactions catalyzed by enzymes are reversible and accumulation of the end products of the reaction may bring the reaction to an equilibrium point at which the hydrolytic reaction and the opposite condensation reaction proceed at the same rate. If the end products are removed, however, the reaction often will continue, usually to completion.

2. *Colloidal Condition.*—Enzymes themselves either exist in a colloidal state or else are so closely associated with other compounds in the colloidal condition as to make it practically impossible to distinguish between the enzyme and the accompanying substances. Willstätter, one of the leading authorities on enzymes, believes them to consist of a small chemically active group bound to a large colloidal carrier. The fact that the most recent chemical analyses indicate that certain enzymes, in as pure a form as it has been possible to prepare them, are either proteins or protein-like compounds probably may be accepted as further evidence of their colloidal condition, since the proteins present in living cells are usually in the colloidal state. Furthermore enzymes dispersed in water exhibit many of the properties of hydrophilic sols, and, like proteins, apparently possess amphoteric properties (Chap. V).

The catalytic effect of enzymes is often interpreted in terms of an adsorption mechanism. By some this association between enzyme and substrate is considered to be a loose chemical combination, by others a true adsorption phenomenon. Whichever of these pictures is considered to represent the relation between enzyme and substrate it is certain that the adsorption—using this term in its broadest sense to refer to either a loose physical or loose chemical combination of a substrate with an enzyme—is a highly selective process.

It is supposed that while the enzyme and substrate are bound together in loose physical or chemical combination that the catalytic effect of the enzyme is exerted. The end products, under the conditions usually prevailing in hydrolytic reactions, are less strongly bound to the enzyme than the substrate, and hence, as in the case of inorganic catalysts, are released into the reaction medium.

3. *Specificity.*—Each enzyme is specific in the sense that it can operate only upon a certain substrate or group of substrates. This does not mean that there must be a separate enzyme for every substrate, but that each enzyme acts only upon substances having a certain molecular configuration. Apparently each individual enzyme can accomplish the dissolution of one particular type of chemical bond. When a number of different compounds possess this bond in common they can be acted upon by the same enzyme. For example the enzyme emulsin can hydrolyze any β-glycoside since the chemical linkage between the sugar and non-sugar groups in all such glycosides is the same.

Enzyme specificity is also illustrated by the fact that different end products are produced from the same substrate under the influence of different enzymes. In the presence of sucrase, for example, the trisaccharide raffinose is hydrolyzed into melibiose and fructose, while in the presence of emulsin the end products of the reaction are sucrose and galactose. Evidently the chemical linkage at which the raffinose is split is different when sucrase is the catalyst than when the reaction proceeds under the influence of emulsin.

4. *Reversibility of Action.*—It has been experimentally shown that a number of hydrolytic enzymes catalyze the same reaction in both directions. The direction which the reaction will go depends upon the relative concentration of end products and substrate, and upon other conditions influencing its equilibrium point. The synthesis of fats from glycerol and fatty acids under the influence of the enzyme lipase has been accomplished experimentally (Chap. XXIII). Similarly, it has been shown that under proper conditions other enzymes such as some of the proteinases and urease can synthesize the compounds which under other conditions serve as their substrates. Glycosides have also been synthesized from the end products of their hydrolysis under the influence of the enzyme emulsin. Hence it is often inferred that all hydrolytic enzymes possess the capacity of catalyzing not only the hydrolysis of a certain substrate, but also, under the proper conditions, the synthesis of that same substrate from the end products of its hydrolysis.

5. *Heat Sensitivity.*—Unlike most inorganic catalysts enzymes are inactivated or destroyed at temperatures considerably below the boiling point of water. At temperatures above 50° C. most enzymes in a liquid medium are rapidly inactivated. Inactivation, although at a slower rate, may also occur at somewhat lower temperatures. Most enzymes in a liquid medium are completely destroyed at temperatures between 60° and 70° C. A few, however, will endure temperatures as high as 100° C., at least for short periods of time. The destruction of enzymes in this range of temperatures is in all probability a heat coagulation phenomenon.

The enzymes of dry tissues such as seeds and spores can endure temperatures of 100° to 120° C., or even higher, for considerable periods without suffering deleterious effects. The same is also true of dried enzyme extracts.

The exact temperature at which a given enzyme will be destroyed varies greatly, depending upon the conditions prevailing in the medium in which it is dispersed. The reaction of the medium has a marked effect upon the heat sensitivity of the enzyme. The presence of either the substrate or the end products of an enzyme in the medium in which it is dispersed greatly retards or may even entirely prevent its destruction at a temperature which would otherwise result in its demolition. The substrate or end products of an enzyme exert a similar protective effect against other destructive agents. An example of this effect, as shown by one of the end products of an enzymatic reaction, is illustrated in Table 44.

TABLE 44—THE ACTION OF FRUCTOSE IN PROTECTING A SUCRASE EXTRACT FROM DESTRUCTION BY ACIDS, ALKALIES, ALCOHOL, AND HEAT (DATA OF HUDSON AND PAINE, 1910)

Concentration of fructose	Destructive agent			
	0.04 *N* HCl, 30° C.	0.03 *N* NaOH, 30° C.	50 per cent alcohol, 30° C.	Distilled water, 61° C.
	Relative rates of destruction			
0 per cent	100	100	100	100
2.7	26	3	1	32
5.4	12	3	1	16
10.9	2	4	1	24

Temperatures near or below the freezing point usually result in the inactivation of enzymes, but not commonly in their destruction. When the temperature is raised to within a suitable range enzymes which have been exposed to relatively low temperatures are generally found to be little if any impaired in their properties.

Occurrence and Distribution of Hydrolytic Plant Enzymes.—The hydrolytic enzymes most commonly found in green plants are amylase, maltase, proteinases, lipases, sucrase, hemicellulases, inulase, emulsin, and the pectic enzymes. Enzymes are very unequally distributed in the various organs and tissues of plants, but there is probably no living cell in which some enzymes, or at least their progenitors, the zymogens, do not occur. Certain organs of a plant apparently contain one set of enzymes, other organs another set. The leaves of the garden beet, for example, are reported to contain

sucrase, maltase and amylase; the stems, sucrase, amylase, inulase, and emulsin, while the roots contain amylase, inulase and emulsion (Robertson, *et al.*, 1909). Germinating seeds are the richest of all plant organs in enzymes and are generally used as the source of enzymes in experimental work.

In some tissues the enzyme and corresponding substrate are both present but only in adjoining cells. This is true, for example, in the seeds of the bitter almond where the enzyme emulsin and its substrate amygdalin both occur but not in the same cells. When bitter almond seeds are crushed the enzyme and its substrate are brought into contact and hydrolysis of the glycoside proceeds (Chap. XXII). More frequently the substrate and the enzyme which catalyzes its hydrolysis are both found in the same cell. Whether or not hydrolysis of the substrate will proceed is dependent upon the conditions which prevail within the cells. It is almost invariably true that plant tissues which are rich in a certain substrate will be relatively rich in the corresponding enzyme, or at least its zymogen, and *vice versa*.

Some plant enzymes, at least, maintain their potency for long periods of time. Miehe (1923) has demonstrated that rye seeds which were at least 112 years old still contained amylase which would hydrolyze starch.

The individual enzymes of most frequent occurrence in plants will now be briefly discussed. The *carbohydrases* will first receive attention.

Amylase (also called diastase[1]) is one of the enzymes of most widespread occurrence in plants. It effects the hydrolysis of starch to maltose through the intermediate stages of the dextrins (Chap. XXII). This enzyme is sometimes considered to consist of a mixture of one or more amylases and one or more dextrinases. Amylase is almost invariably present in the green parts of plants, and is usually found in non-green organs or tissues as well. This enzyme is also produced by many bacteria and fungi, as well as by most animals.

Commercial diastase is prepared from germinating barley seeds (malt) in which the enzyme was first discovered. Takadiastase, another commercial form of this enzyme, is prepared from colonies of a mold (*Aspergillus oryzae*) which has been allowed to grow on a substrate of steamed wheat bran or rice.

It is generally considered that there are two well-defined types of plant amylase or diastase, the so-called "secretion diastase" and the so-called "translocation diastase." The former of these is secreted by specialized cells and is only known to occur in the grains of corn, wheat, barley, and other members of the grass family (Brown and Morris, 1890). "Translocation diastase," on the other hand, is widely distributed in plant tissues and is especially

[1] This term is also sometimes applied to enzyme extracts which consist of a mixture of amylase and maltase.

abundant in green leaves and actively growing plant parts. It is so named because it is the form of diastase which hydrolyzes the starch in leaves before it is translocated to other parts of the plant.

Maltase is the enzyme which catalyzes the digestion of maltose to glucose according to the following equation:

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Maltase}} 2\ \underset{\text{Glucose}}{C_6H_{12}O_6}$$

Maltase is one of the most widely distributed of the plant carbohydrases and is almost invariably found to be present in tissues which contain amylase. It is also produced by many species of micro-organisms and is found in various organs of the higher animals. This enzyme can also hydrolyze α-glycosides. Maltose itself is an α-glycoside of glucose with glucose.

Sucrase (*invertase*) catalyzes the hydrolysis ("inversion") of sucrose to hexose sugars according to the well known equation:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Sucrase}} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

This is another enzyme of widespread occurrence throughout the plant and animal kingdoms. It has been found in practically all of the green plants which have been examined for its presence, occurs in the bodies of the higher animals, and is produced by many micro-organisms. Sucrase is synthesized in great abundance by many of the yeasts, hence these organisms are generally used as a source of this enzyme.

Since the hydrolysis of sucrose is a relatively simple reaction, involving well-known products, and can be followed very simply by means of a polarimeter (Chap. XXII), it has been studied more comprehensively than any other process catalyzed by enzymes. Most of the studies of the effect of various factors upon the velocity of enzyme reactions have been made with this enzyme. Its only rival as a favorite in such studies is catalase (Chap. XXX).

Inulase is the enzyme which catalyzes the hydrolysis of inulin to fructose, according to the equation:

$$\underset{\text{Inulin}}{(C_6H_{10}O_5)_n} + n\,H_2O \xrightarrow{\text{Inulase}} n\ \underset{\text{Fructose}}{C_6H_{12}O_6}$$

This enzyme appears to be of rather limited occurrence. It has been found in the tubers of the Jerusalem artichoke (*Helianthus tuberosus*) and in several other species which accumulate inulin. It is also produced by certain fungi (*Aspergillus niger* and *Penicillium glaucum*), and in some animals, especially of the invertebrate group.

Three rather distinct pectic enzymes are generally recognized: *protopectinase, pectase,* and *pectinase.* Protopectinase catalyzes the reaction by which protopectin is converted into pectin. Pectase, which can properly be classed among the esterases, results in the hydrolysis of pectin to pectic acid and methyl alcohol, while pectinase results in the hydrolysis of pectin, pectates or pectic acid to arabinose, galactose, and galacturonic acid. The chemical aspects of these various transformations among the pectic compounds have already been discussed in Chap. XXII.

Protopectinase is found both in the higher plants and in certain parasitic fungi. Pectase has been found in a large number of species of plants, and seems to be especially abundant in the leaves. Pectinase, like protopectinase, is found principally in the fungi, although it is not entirely unknown in the higher plants. Certain fungi are apparently able to penetrate the middle lamella of plant cells by secreting this enzyme and digesting the constituent pectic compounds.

Hemicellulases (cytases) are enzymes which accomplish the hydrolysis of the various hemicelluloses into hexoses and pentoses. The presence of hemicellulase has been demonstrated in the endosperm of the seeds of various species, especially those in which hemicelluloses are relatively abundant. Hemicellulases are also found in some species of bacteria and fungi, and in some invertebrate animals, including certain insects.

Cellulase, the cellulose digesting enzyme, results in the hydrolysis of cellulose into cellobiose (Chap. XXII), a reaction analogous to that occurring in the digestion of starch to maltose. Cellobiose is further hydrolyzed by the enzyme *cellobiase* to glucose. These reactions are as follows:

$$\underset{\text{Cellulose}}{(C_6H_{10}O_5)_n} + n\,H_2O \xrightarrow{\text{Cellulase}} n\,\underset{\text{Cellobiose}}{C_{12}H_{22}O_{11}}$$

$$\underset{\text{Cellobiose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{Cellobiase}} 2\,\underset{\text{Glucose}}{C_6H_{12}O_6}$$

Cellulase has not been isolated from the higher plants, but is found in certain bacteria and fungi, particularly those species responsible for the decay of wood, straw, leaves and other cellulose-containing plant residues. Some of the higher animals, particularly those of the herbivorous group, apparently can also digest cellulose, but this has been found to be due to the presence in the digestive tract of certain species of cellulose-decomposing bacteria.

Emulsin or β-glycosidase catalyzes the hydrolysis of any of the β-glycosides such as amygdalin, salicin, phloridzin and arbutin (Chap. XXII). It is not a single enzyme, but a mixture of at least two—*amygdalase* and *prunase.* Emulsin is widely distributed in the plant kingdom. It is especially abundant

in the seeds of both sweet and bitter almonds and various organs of other members of the *Rosaceae*. Emulsin is also found in some fungi and invertebrates, but is not known to occur in any of the higher animals.

The term *esterases* is applied to the general group of enzymes that catalyze the hydrolytic splitting of esters into simpler compounds. The most important sub-group of the esterases are the fat-hydrolyzing enzymes or *lipases*. The term lipase is generally used in a collective sense in speaking of the enzymes of this group, as it is by no means certain whether there is only one lipase or a number of different lipases. The latter view is perhaps more generally held. Lipase is known to catalyze both the digestion of fats to fatty acids and glycerol as well as the synthesis of fats from these two compounds. Lipase is present in greatest abundance in germinating seeds containing relatively large quantities of fats, such as castor bean, soy bean, coconut, flax, hemp, rape, and corn. Its presence can also be demonstrated in the resting seeds of some, but not all, of these species. In those in which it cannot be demonstrated in the seeds prior to germination it is believed to be present in the form of a zymogen. This enzyme is also found in animals and in some species of bacteria.

As shown in Table 43 three different kinds of proteinases are recognized (Grassmann, 1932). Of these the papainases are the most common in plants. *Papain* is the best known of this group of enzymes. It occurs throughout the tissues of the tropical papaw (*Carica papaya*) a species native to Central and South American regions, but now cultivated in many other parts of the world, including southern Florida. Natives of regions where this plant grows have long known that its leaves have a digestive effect on meat. It is said that simply wrapping meat in crushed papaw leaves will increase its tenderness. Commercial preparations of papain are prepared from the latex of this species in which it is especially abundant. Incisions are made in the plant, the milky exudate is collected, dried, and pulverized, and in this form becomes the "papain" of commerce.

Bromelin is a papainase which occurs in considerable quantities in the pineapple (*Ananas sativa*) and other members of the *Bromeliaceae*. A crude extract of bromelin can be obtained by squeezing out the juice of a fresh, ripe pineapple. By suitable treatments purified and concentrated extracts of this enzyme can be prepared.

Reactions catalyzed by proteinases generally proceed at a much slower rate than most other enzymatic reactions catalyzed by plant enzymes, at least under laboratory conditions. An amylase extract which will hydrolyze a fairly concentrated starch sol in a few minutes can be easily prepared but hydrolysis of proteins by proteinases requires from several hours to several

days. For this reason it is more difficult to demonstrate the presence of proteinases in plant tissues than other enzymes and more difficult to study their action even after suitable extracts have been prepared.

Many plant enzymes are classified as proteinases which more properly come under the heading of peptidases (Table 43). Following an older terminology such enzymes are often called *erepsins,* and have been found to be of wide distribution in plants.

Synthetic Action of Enzymes.—In the foregoing discussion our attention has been directed to the hydrolytic action of enzymes. Many and perhaps all hydrolytic enzymes also catalyze the corresponding condensation reaction. The rôle of enzymes in the synthetic reactions of plants should be stressed, although it has been found much more difficult to study this phase of their activity experimentally than their hydrolytic effects. Probably all of the condensation reactions involved in the synthesis of the many types of carbohydrates, lipids, and proteins in plants proceed under the influence of enzymes. It is doubtful, however, if there is actually a specific enzyme for every reaction which occurs in a living organism, as is sometimes stated. However, that the key reactions occurring in any organism—those which determine the metabolic pattern of that organism—proceed under the controlling influence of enzyme catalysis seems beyond doubt.

Factors Affecting Enzymatic Reactions.—The influence of various factors upon the reactions of the enzymes of the higher plants has been studied almost entirely *in vitro.* The usual procedure has been to prepare a more or less purified extract of the enzyme to be studied, then to bring this extract into contact with the substrate under controlled conditions, and finally to determine the rate of the ensuing reaction. Enzymatic reactions as they occur in living cells are influenced by conditions within the protoplasmic matrix, and in this respect differ very greatly from such reactions occurring *in vitro.* Since the conditions which influence the action of an enzyme in an artificial medium are usually very different from those obtaining in a living cell, it is certain that the effects of various factors on extracted enzymes are quantitatively unlike those which they would have on that same enzyme *in vivo,* although qualitatively the effects are undoubtedly very similar.

1. *Temperature.*—The rate at which an enzymatic reaction will proceed is influenced not only by the temperature, but also by the length of time which the reaction mixture has already been at that temperature. In other words, as in the process of photosynthesis, a "time factor" must be recognized in considering the effects of temperature upon the rate of an enzymatic reaction. In general the initial velocity of enzymatic reactions is accelerated with increase in temperature until a certain "optimum" is attained. The

initial velocity of the reaction may be maintained almost indefinitely at lower temperatures, but the higher the temperature, the more markedly it decreases with time.

The optimum temperature for most enzymatic reactions, if measured in terms of hours, lies between 40° and 50° C., although for a few it is as high as 60° C. If only a very short interval of time is allowed to elapse before a determination of the amount of substrate hydrolyzed is made, a higher optimum will usually be found, than if longer time intervals are used as a basis of measurement. The optimum is influenced, however, not only by the time factor, but also by other conditions prevailing in the medium such as the pH, relative concentration of enzyme and substrate, etc.

At temperatures above the optimum point for any enzymatic reaction, inactivation of the enzyme occurs so fast that the rate of the reaction is rapidly decreased. It is also possible that the retarding influence of relatively high temperatures may sometimes be due to a greater acceleration of the reverse reaction (whereby the substrate is resynthesized from the end products) than the hydrolytic reaction.

At temperatures below those at which an enzyme is rapidly inactivated a 10° C. rise in temperature usually increases the rate of the reaction catalyzed by that enzyme from two to three times. In other words the temperature coefficient (Q_{10}) of an enzymatic reaction is generally between two and three.

2. *Hydrogen Ion Concentration.*—Sorensen, who first proposed the pH system of notation as a method of indicating hydrogen ion concentration, was one of the first workers to realize clearly that the speed of reactions catalyzed by enzymes is greatly influenced by the pH of the medium in which the reaction occurs. This is one of the most important known effects of hydrogen ion concentrations in the realm of biological phenomena. This fact fits in very well with the generally held presumption that enzymes are amphoteric substances. The hydrogen ion concentration of the medium exerts a controlling effect on the sign and the magnitude of the charges carried by the particles of ampholytes. Apparently the activity of an enzyme is at its maximum when it carries a certain charge and inactive when the charge deviates too far from the value in either direction. Hence the pH value at which a charge of the proper magnitude and sign is present on the enzyme is the one at which the enzyme will operate at its maximum efficiency. The pH of the medium may also influence the charge on the substrate as well as the charge on the enzyme. The influence of hydrogen ion concentration on enzymatic activity may be due partly to this effect.

In general there appears to be an optimum hydrogen ion concentration

for the activity of each enzyme, as well as upper and lower limits of hydrogen ion concentration beyond which the enzyme is inactive, or may actually be destroyed. The optimum hydrogen ion concentration for the activity of any enzyme may vary, however, depending upon the source of the enzyme, temperature, length of time the enzyme has been kept under any particular set of conditions, etc. Typical curves for the relation between pH and the activity of the enzyme amylase are shown in Fig. 101. Curves expressing this relationship for other enzymes follow somewhat similar trends, but the optimum and limiting pH values vary considerably. Most such curves indicate that relatively small shifts in pH value from the optimum result in marked changes in the activity of the enzyme.

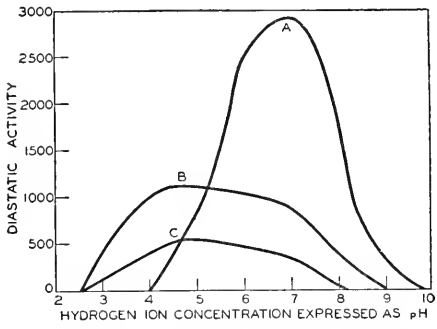

FIG. 101. Relation between hydrogen ion concentration and diastatic activity. (*A*) pancreatic amylase, (*B*) amylase of malt, (*C*) amylase of *Aspergillus oryzae*. Data of Sherman, *et al.* (1919).

In general most hydrolytic plant enzymes exhibit their maximum activity in an acid medium—usually between pH 4.0 and 7.0. Most of the oxidizing-reducing enzymes (Chap. XXX), on the other hand, appear to attain their maximum activity in neutral or alkaline solutions.

3. *Hydration.*—The effect of increased hydration upon the enzyme activity of plant tissues is most easily demonstrated during the germination of seeds. The enzyme activity, measured in terms of any specific enzyme known to be present, is usually low in dry but viable seeds. As imbibition of water proceeds during germination the activity of the enzyme increases more or less progressively, an effect which can be ascribed, at least indirectly, to the increase in the hydration of the tissues of the seed (Pickler, 1919). Sometimes, however, this apparent increase in the activity of an enzyme with increase in the hydration of the tissues during seed germination can be ascribed to the conversion of zymogens into enzymes. Similar effects upon enzyme activity are undoubtedly shown in other plant tissues in which considerable changes in hydration occur.

4. *Concentration of the Enzyme.*—Since, with a few possible exceptions, the chemical nature of enzymes is unknown, the concentration of an enzyme extract usually cannot be determined or stated in any absolute terms. The effects of *relative* concentrations of an enzyme extract upon the rate of

enzymatic reactions can, however, be readily determined. It is only necessary to dilute a given extract to one-half, one-fourth, one-eighth, etc. of its initial concentration and to study the rates of reaction resulting when a series of such diluted extracts is employed. In general the activity of extracts of most enzymes is approximately proportional, at least within limits, to the concentration of the enzyme (Fig. 102).

5. *Concentration of the Substrate.*—The velocity of an enzymatic reaction usually increases with increase in the concentration of the substrate up to a certain maximum, after which the relative amount acted upon per unit of time may decrease with increase in the substrate concentration. The retarding effect of relatively high concentrations of the substrate upon enzyme activity may be due in part to the more rapid accumulation of the end products of the reaction (see next section). Furthermore as the concentration of the dissolved or dispersed substrate in a reaction mixture increases, the concentration of water decreases. The smaller proportion of water present at higher substrate concentrations prevents the action of the enzyme at its maximum efficiency.

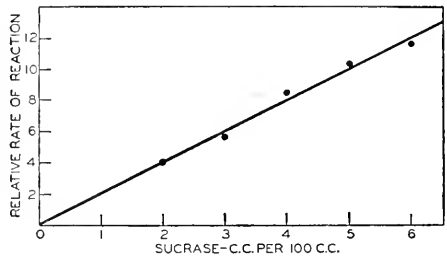

FIG. 102. Relation between concentration of sucrase and its hydrolytic activity. Data of Nelson and Hitchcock (1921).

6. *Concentration of the End Products.*—Enzymatic reactions, like all other chemical reactions, are subject to the laws of chemical equilibrium and mass action. Hence as the end products of the reaction accumulate the apparent rate of the reaction decreases. Similarly if the concentration of the end products in the reaction mixture is initially high the rate of the reaction will be slower than if the end products are lacking. In some enzymatic reactions the enzyme itself combines with one of the end products. This results in a reduction in the active concentration of the enzyme present, and since the rate of the reaction is proportional to the active concentration of the enzyme, its velocity is slowed down.

7. *Accelerators.*—Certain specific chemical substances are known to exert a stimulating effect upon enzyme activity. Such substances are generally called *accelerators* or *activators.* Some accelerators seem to be general, *i.e.* increase the activity of all or most enzymes, while others are specific for certain enzymes. Among the former are low concentrations (2-5 per cent) of the salts of the alkali and alkaline earth metals. Accelerators may exert their influence either by some direct effect upon the enzyme, or by eliminating the

effect of some substance or factor which inhibits the operation of the enzyme. Specific enzyme accelerators are generally called *co-enzymes*. The enzyme zymase (Chap. XXX), for example, will not operate unless two co-enzymes are present in the same medium with it.

8. *Inhibitors.*—Substances in the presence of which enzymes are rendered inactive, or at least markedly reduced in activity, are termed *inhibitors* or *paralyzers*. The effect of certain inhibitors may be due to the actual destruction of the enzyme, of others to some effect upon the substrate that renders it less readily susceptible to the action of the enzyme, and of others to an influence upon the velocity of the enzymatic reaction. The salts of most of the heavy metals (Au, Ag, Hg, Cu, Ni, Co, etc.) act as very effective inhibitors of most enzymatic reactions. Salts of the alkali and alkaline earth metals, in high concentrations, also usually exert an inhibiting effect upon the action of enzymes.

Various organic substances also inhibit enzymatic reactions. Among these are hydrocyanic acid and formaldehyde. Chloroform also inhibits enzymatic reactions but its retarding effect varies considerably with the specific enzyme. Some of the other common organic reagents such as acetone, toluol, and ethyl ether exert little or no effect upon the activity of enzymes. Ethyl alcohol in solutions of approximately 50 per cent concentration is very destructive to enzymes but as its concentration is increased or decreased from this value its deleterious effect becomes progressively less marked. Protoplasm is generally more sensitive to the effects of such organic reagents as chloroform, ether, etc. than enzymes. Hence it is often possible to kill plant cells by suitable treatment with such reagents without destroying the enzymes within them. Similarly cells of most plant tissues can be killed by desiccation or freezing without seriously affecting the properties of the enzymes present.

There is considerable evidence that living organisms themselves produce substances that inactivate enzymes. Such substances are termed *anti-enzymes* and can be classed as naturally produced enzyme inhibitors.

9. *Radiant Energy.*—Certain wave lengths of radiant energy exert very pronounced effects upon the activity of enzymes *in vitro*. Ultraviolet rays usually have a marked inactivating effect upon enzymes. The shorter wave lengths of the visible spectrum seem to have greater inactivating effect than the longer wave lengths. It has sometimes been assumed that the well known effect of the shorter wave lengths of light in checking growth rates of plants is due, at least in part, to such an effect on the enzymes of the meristematic cells, but there is practically no valid evidence in support of this hypothesis.

Chemical Nature of Enzymes.—It is evident that in any ultimate analysis enzymes must represent chemical compounds of definite molecular structure and configuration, and that these molecules, whether they exist in the cells in true solution, in colloidal dispersion, or as adsorbed substances in a colloidal complex, must contain certain chemical groupings which can accomplish their characteristic catalytic effects. It has long been suspected, both because of their intimate relation to protoplasm, and their apparent colloidal properties, that enzymes are either proteins or protein-like substances. At the present time evidence is rapidly accumulating in support of the view that many enzymes, at least, are proteins.

Sumner's work on urease (1926, 1928) was the first to point in this direction. Urease is the enzyme which catalyzes the hydrolysis of urea, as follows:

$$\underset{\text{Urea}}{(NH_2)_2CO} + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

This enzyme is produced by many bacteria and fungi, and is also found in a number of species of the higher plants. Sumner succeeded in isolating from the seeds of the jack bean (*Canavalia ensiformis*) a crystalline protein which he regards as the enzyme urease in pure form. This product was crystallized out of acetone extracts of the ground seeds. His preparations exhibit activities of from 730 to 1400 times that of the original material, and appear to have the properties of a globulin.

Recently a number of other enzymes have apparently been isolated in a pure form. Among these are pepsin (Northrup, 1930), trypsin (Northrup and Kunitz, 1932), amylase (Sherman, *et al.*, 1931), and papain (Balls, *et al.*, 1937). All of these enzymes in the isolated form appear to be crystalline proteins.

It would be premature to conclude, however, that all enzymes are proteins or protein-like compounds. In fact there is considerable evidence that certain ones such as the oxidizing-reducing enzymes catalase and peroxidase (Chap. XXX), or at least their active groups, are non-proteins. It is entirely possible that some enzymes may be proteins while others are not.

The Production of Enzymes by Plants.—It has already been pointed out that plant enzymes are mostly of the endoenzyme type which means that they generally operate within the cells in which they are produced.

There are only two well-known examples of the formation of exoenzymes by the higher plants. One of these is in the so-called insectivorous plants such as certain pitcher plants and sundews. The leaves of these species

have been shown to secrete proteinases into the liquid in the "pitcher" or, in the sundews, on the surface of the leaf.

Certain tissues of the embryo in the grains of various members of the grass family also produce exoenzymes (Brown and Escombe, 1898, and others). This phenomenon cannot be appreciated without a knowledge of the various parts of the grain type of fruit. These are illustrated in Fig. 103 which represents a longitudinal section cut through a corn grain across the shorter of the two transverse axes. The structure of the other grains

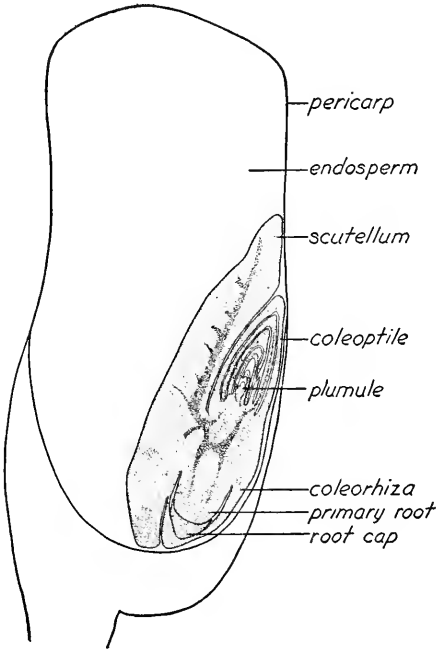

FIG. 103. Longitudinal section through a corn (maize) grain to show principal parts of grain and embryo.

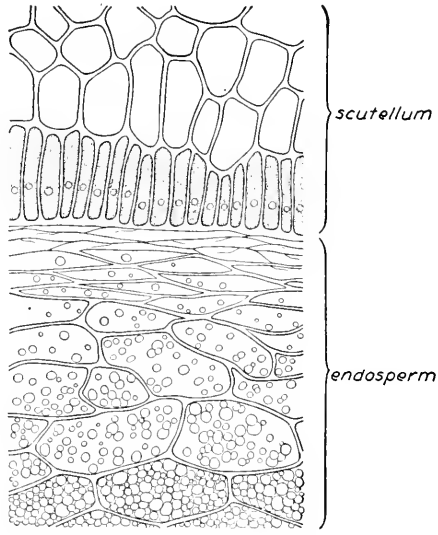

FIG. 104. Section through a small portion of a germinating corn (maize) grain. Starch has been digested in several layers of endosperm cells adjacent to the scutellum.

(wheat, barley, rye, etc.) is, in general, very similar to that of the corn grain.

Shortly after a grain of corn, barley, or one of the other closely related species begins to imbibe water striking changes take place in the appearance of the epithelial cells of the scutellum (Fig. 104). The protoplasmic contents of these cells, originally finely granulated and nearly transparent, become much coarser in texture and clouded in appearance. The nucleus, originally clearly visible, is obscured by this change in the physical properties

of the cytoplasm. The epithelial cells also swell to several times their original volume during the first few days of germination.

Not long after these changes occur in the appearance of the cells of the epithelium the cell walls of the endosperm cells adjacent to the epithelial layer begin to disappear, and the starch grains in the cells of the endosperm begin to show corrosion. Shortly afterwards transitory starch grains appear in the parenchymatous cells of the scutellum. Enzymes produced in the layer of epithelial cells apparently have diffused into the endosperm and there set in operation digestive processes, the products of which subsequently diffuse into the scutellum. This seems to be the mechanism whereby the embryo obtains food from the endosperm during the process of germination. The principal enzymes secreted by the embryo are hemicellulase which results in the dissolution of the endosperm cell walls, and diastase (amylase plus maltase) which results in the digestion of starch. Similar phenomena undoubtedly occur during the germination of other kinds of seeds.

Suggestions for Collateral Reading

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CHAPTER XXVIII

TRANSLOCATION OF SOLUTES

In most plants a large proportion of the living cells do not contain chloroplasts. All such non-green cells are dependent for essential carbohydrates upon the chlorophyllous cells of the plant. Many of the non-green cells are remote from the photosynthesizing cells. The cells in the root tips of trees, for example, are sometimes hundreds of feet distant from the nearest leaves. They, as well as all other non-green cells in the body of a plant, are dependent for their existence upon the simple carbohydrates which are carried to them through intervening tissues from the chlorenchyma. The movement of solutes such as simple carbohydrates from one part of a plant to another is designated as the *translocation, transport,* or *conduction* of solutes. Many other kinds of solutes are translocated in plants besides carbohydrates, and transport occurs in upward and lateral directions as well as in a downward direction.

Cell to cell movement of solutes may occur in any part of a plant but the term translocation is generally restricted to the movement of solutes in the specialized tissues of the phloem and xylem in which the distance through which they are transported is usually very great in proportion to the size of the individual cells.

Anatomy of Phloem Tissues.—Of the various stem tissues only the xylem and phloem possess such a structure as to suggest that a relatively rapid longitudinal movement of solutes can occur through them. Both of these tissues are characterized by the presence of elongated cells and elements which are joined in such a way as to form essentially continuous ducts. Furthermore, it has been shown experimentally that the rate of movement of solutes through other stem tissues such as the pith and cortex is totally inadequate to account for known rates of translocation through stems.

The structure of the xylem tissues has already been discussed in Chap. XV. Discussion of the anatomy of the conductive tissues will now be completed by a consideration of the structure of the phloem. Like the xylem the phloem is continuous from the top to the bottom of the plant, the ultimate terminations of the phloem system being in the tissues of the stem

tips or lateral organs and in the root tips. Although in most species solutes move for the greatest distances through stems it is important to realize that the conductive tissues of plants constitute a complicated but unit system which ramifies to all parts of the plant body.

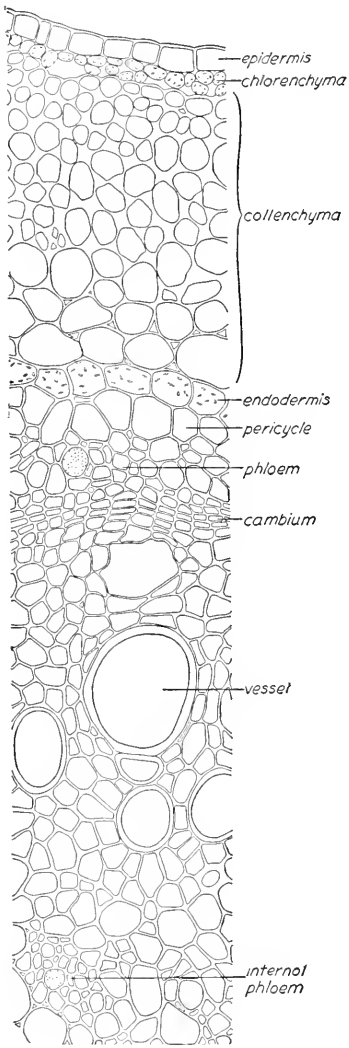

FIG. 105. Cross section of a small portion of a tomato stem showing internal phloem.

The general arrangement of the xylem and phloem tissues in representative types of stems has already been considered (Fig. 53, Fig. 54). In the majority of dicot stems the phloem usually occurs as a continuous cylinder of tissues just external to the cambium layer. In a few species some strands of internal phloem are present inside of the xylem (Fig. 105). In roots the primary phloem and xylem are present, as seen in cross section, in a radial arrangement, but the secondary conductive tissues are oriented in essentially the same pattern found in stems (Chap. XVII).

Five principal types of cells are found in the phloem: (1) *sieve tubes*, (2) *companion cells*, (3) *phloem fibers*, (4) *phloem parenchyma*, and (5) *phloem ray cells*. The proportions of these various types of cells present are different in every individual species and in some species not all are present.

Mature sieve tubes consist of linear series of elongated, rather thick-walled cells which are joined together end to end (Fig. 106). The individual cells are also often referred to as sieve tubes, but a better term for them is *sieve tube elements*. The cross walls separating the cells composing the sieve tubes are often oblique. Strands of cytoplasm pass from one sieve tube element to the next through pores in the end walls. These pores are grouped into definite areas called *sieve plates*. Sieve plates often are also present in the side walls of the sieve tubes. In many species the end walls of the sieve tube elements are one large sieve plate. In

potato stolons the sieve tube elements range in length up to 100 μ, while in cucurbits some of them attain lengths of 1000 μ, but in most species they are shorter.

Most of the protoplasm of sieve tube elements is present as a thin layer between the central vacuole and the cell wall. In mature elements fine strands

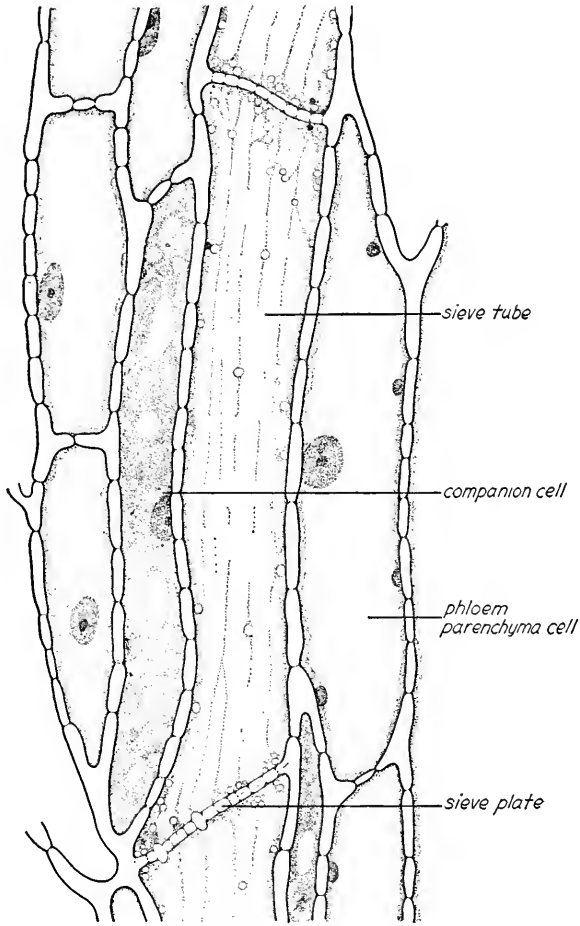

FIG. 106. Longitudinal section of a sieve tube and associated cells from the stem of tobacco. Redrawn from Holman and Robbins (1938).

of protoplasm cross the vacuole. Cytoplasmic connections are established between adjacent elements through the intervening end walls as the element matures. Young sieve tube elements contain one or more nuclei, but as the

elements mature the nuclei enlarge and disintegrate. The vacuolar sap of sieve tube elements contains conspicuous amounts of proteinaceous colloidal material. In mature sieve tubes the cytoplasm seems to be in a highly permeable condition to both water and solutes.

Sieve tubes usually develop by the longitudinal division of sieve tube mother cells which have arisen as a result of the division of a cambium cell. The division of such sieve tube mother cells results in a sieve tube element and a *companion cell* (Fig. 106). Sometimes the sieve tube mother cell may divide longitudinally more than once producing one sieve tube element and two or more companion cells. The companion cells are much smaller in cross section than the sieve tube elements. They may have the same length as the sieve tube element or they may be half or even less its length.

The protoplasm of each companion cell contains a prominent nucleus, and numerous small vacuoles, but no starch grains. The walls separating the companion cell or cells from the sieve tube element are characterized by the presence of numerous small pits but the walls between the companion cells and the parenchyma cells contain few or no pits. The presence of these pits suggests a close relationship between the companion cells and the sieve tube elements. In some species some sieve tube elements do not have companion cells adjacent to them while in other species every sieve tube element is accompanied by one or more companion cells. Gymnosperms and pteridophytes do not have companion cells.

Phloem parenchyma is composed of living cells which are somewhat elongate parallel to the long axis of the stem. This tissue does not occur in monocots and is also absent from the phloem of some dicots. The proportion of phloem parenchyma cells in the phloem varies widely according to species. In most herbaceous dicots they constitute a smaller proportion of the phloem than do the sieve tubes and companion cells. The phloem of seedlings, however, may consist largely of phloem parenchyma. Woody species also differ greatly in the proportion of phloem parenchyma cells present. The arrangement of phloem parenchyma cells likewise exhibits a great variation. They may occur in definite clusters, in tangential bands, or in radial rows that are closely associated with the sieve tubes. Many of the phloem parenchyma cells store starch and in certain species they often contain crystals.

Phloem fibers, found in the phloem of some plants, are elongated cells with thick, usually lignified walls. They are more common in woody than in herbaceous species and, like the companion cells, they do not occur in the gymnosperms or in the pteridophytes. The phloem fiber cells have long, tapering ends which overlap forming strong fibrous strands. They frequently occur in groups or as cylindrical sheaths surrounding the inner phloem tissues.

As discussed in Chap. XV vascular rays are present in the stem tissues of most species. The vascular rays are initiated in the cambium and extend both into the xylem and into the phloem. The distribution of the *phloem rays,* as the portions of the vascular rays located in the phloem are called, varies greatly according to species. As a rule, they consist of band-like bundles of transversely oriented living cells varying from one to many cells in width and from several to many cells in height. Certain types of phloem ray structure are characteristic of each species. The cells of phloem rays are radially elongate, and parenchymatous. The phloem ray cells of roots and stems store considerable quantities of starch and probably other organic compounds as well.

Longitudinal conduction in the phloem undoubtedly occurs principally through the sieve tubes, since they alone are universally present and are so constructed that they offer less resistance to movement than other types of phloem cells. Nevertheless there is a distinct possibility that the parenchyma and companion cells also play a part in translocation processes. The phloem ray cells undoubtedly serve as channels of lateral conduction between the phloem and xylem. The phloem fibers probably play no part in conduction.

The functional life of the sieve tubes is relatively short although in annuals they may serve as channels of conduction for the entire life of the stem. In many perennial stems the protoplast disappears from the sieve tubes by the end of the first growing season, and in few species do they remain alive for more than several years. Although it is generally believed that the sieve tubes continue to serve as routes of translocation as long as the protoplast is present, some authorities consider that their functional activity terminates with the disintegration of the nucleus which occurs relatively early in their life history. In woody stems the phloem parenchyma and phloem ray cells usually remain alive much longer than the sieve tubes.

As the sieve tubes age the sieve plates often become capped with masses of callose (Chap. XXII). These carbohydrate plugs are known as *callus,* and apparently largely or entirely prevent translocation through the sieve tubes. In some woody species temporary caps of callus are deposited at the approach of the dormant season, only to be dissolved when growth activity is resumed. In many species permanent callus caps sooner or later develop on the sieve plates. It is generally believed that such permanent callus develops at the time that the functional activity of the sieve tubes ceases.

Continued formation of new layers of xylem and phloem ultimately results in most species in crushing the older, now dead, phloem tissues. The changes which occur as phloem grows older include lignification of fibers and sometimes also ray and parenchyma cells, and, in certain species, modification

of ray and parenchyma cells into hard, thick-walled, dead cells known as *stone cells*. In woody stems further profound modifications occur in the aging phloem tissues as the result of the activity of secondary meristems called *cork cambiums*.

Downward Translocation of Organic Solutes.—Downward translocation of organic solutes unquestionably occurs for the most part through the phloem tissues. The only possible alternative theory, that downward translocation occurs in certain xylem elements, has been advocated occasionally and this possibility will be examined briefly in the discussion.

Most of the evidence indicating that organic solutes (principally carbohydrates) move toward the basal portions of plants in the phloem has been obtained by ringing experiments. "Ringing," when the term is employed without qualification, refers to the removal of a narrow continuous band of the tissues external to the xylem. Since the ringing entirely encircles the stem all tissues external to the xylem are completely intercepted. This operation is also often called "girdling." Ringing is seldom practiced except on woody stems in which it is equivalent to removing a strip of bark and destroying the cambium.

In a girdled tree carbohydrates slowly accumulate in the tissues above the ring, while the quantity of carbohydrates in the stem and root tissues below the ring slowly become depleted as a result of their utilization in respiration and assimilation. Such a result of girdling has also been demonstrated in certain herbaceous plants such as cotton (Mason and Maskell, 1928).

This indicates that the normal downward translocation of carbohydrates occurs through the phloem tissues. Such a conclusion is also substantiated by experiments in which the cortical tissues external to the phloem are removed but the phloem itself is left intact. Downward translocation is not appreciably influenced by treating plants in this manner.

Horticulturists often practice temporary ringing of the branches of apple trees in order to increase the size of developing fruits. Temporary ringing is accomplished by cutting out such a narrow strip of bark that the gap is gradually bridged by wound tissue. The interception of the phloem persists long enough, however, to interrupt temporarily the movement of soluble carbohydrates to lower parts of the tree. Carbohydrates synthesized by the leaves on the ringed branch mostly remain in that branch or are translocated to the developing fruits. The greater supply of foods favors enhanced growth of the fruits.

Chemical analyses show that the cells of the phloem are relatively rich in carbohydrates and organic nitrogenous compounds. This finding is in accordance with the concept that translocation of organic compounds occurs

through the phloem, but is not in any sense a proof, since storage tissues also contain relatively high concentrations of foods.

Another reason frequently advanced for considering the phloem as the tissue which is primarily concerned in the downward translocation of organic solutes is that the general direction of the movement of sap in the xylem is upward. It is not inconceivable, however, that there might be descending streams in certain xylem ducts, even while the majority are occupied with upward moving columns of water. In fact theories of the downward translocation of solutes involving this very concept have been advanced from time to time, although currently they find little support. If a dye is injected into the xylem of a woody stem it usually moves both upward and downward. This has sometimes been cited as evidence of downward currents in the xylem. Such downward movements of injected dyes are probably due to the effects of tension or to the entrance of the dye into vessels which are occupied only by gases at a subatmospheric pressure, and is scarcely proof that downward movements normally occur in the xylem of intact stems.

While it is possible that backward movement of liquid may occasionally occur through the xylem, all the evidence indicates that this tissue plays no important rôle in the normal downward conduction of solutes in plants.

Upward Translocation of Organic Solutes.—Under many conditions an upward [1] translocation of organic solutes takes place in plants. This occurs, for example, in the stems of woody species when the buds resume growth in the spring. The tissues of the new shoots are constructed largely out of foods which move in an upward direction from the storage tissues of the stems, as during the early stages in their expansion the leaves do not photosynthesize at a rate sufficient to supply all the foods used in the growth of the shoot which bears them. Upward translocation of foods from the older leaves on a given shoot to developing leaves situated closer to its apex also probably occurs. As the leaves mature there is a reversal in the direction of translocation of carbohydrates; they are then translocated from the leaves into the stems in which they generally move in a downward direction.

A number of other examples of the upward transport of foods in plants can be cited. Developing fruits are often attached to stems in such a position that some or all of the food translocated into them moves through the stems in an upward direction. In the early stages of the development of seedlings upward translocation occurs from the endosperm or cotyledons towards the

[1] The terms "upward" and "downward" as applied to translocation phenomena should not be interpreted too literally. As a rule translocation in the general direction of roots to leaves or other apical regions is termed "upward translocation"; movement in the reverse direction, "downward translocation."

apical portions of the plant in which rapid growth is taking place. Likewise upward transport of foods invariably occurs during the earlier stages of shoot growth from bulbs, tubers, rhizomes, and other types of underground organs.

The "classical" view that upward translocation in plants takes place in the xylem has generally been accepted, at least until comparatively recently, as referring to mineral salts and organic solutes as well as to water. The concept that upward transport of carbohydrates occurs principally through the xylem is based largely on phenomena which have been observed in certain woody species. Large quantities of soluble and insoluble carbohydrates are stored in the wood parenchyma, wood rays, and (in the younger stems) pith cells of many varieties of trees and shrubs. At certain seasons soluble carbohydrates are also found in the xylem conduits, as illustrated by data of Anderssen (1929, Fig. 107). As found by this investigator the concentrations of both sucrose and free reducing substances (probably largely hexoses) in the tracheal sap of pear trees were highest in the winter and early spring. Both fell to a zero value during the summer months, and increased slowly during the autumn. The sugars found in the conducting elements undoubtedly come from the storage tissues of the pith or xylem. The relatively high soluble carbohydrate content of the xylem tissues in the winter and spring is probably due largely to shifts in the starch $\rightleftarrows$ sugar equilibrium towards the sugar side, as a result of the relatively low temperatures prevailing during these seasons (Chap. XXII).

Since in the early spring the soluble carbohydrate content of the sap is relatively high, and by the time the shoots are well developed has dropped to a low or zero value, this seems to indicate that soluble carbohydrates have been conducted through the xylem up to the developing buds. Although this assumption appears to be superficially plausible there are good reasons for doubting if this phenomenon actually is very strong evidence that upward translocation of organic solutes does occur in the xylem. In the first place the concentration of organic solutes in the xylem sap is always very low. In the sugar maple the proportion of sugar in the sap of the vessels may attain 8 per cent, but is usually less and in most other species seldom exceeds 2 per cent. Furthermore the highest concentrations of sugar occur in the sap during the winter when there is little or no upward translocation of water, and it is not at all certain that these solutes do not largely disappear from the xylem sap in the spring before its upward flow begins to take place at an appreciable rate.

Recent investigations by Curtis, summarized in 1935, point to a conclusion regarding the upward transport of organic solutes which is directly the opposite of the long accepted view. His experiments all seem to indicate

that it is the phloem and not the xylem which is the principal tissue through which such translocation occurs. In one of the most convincing of his experiments the contrasting effects of intercepting the xylem and intercepting the phloem of woody stems upon upward transport of organic solutes was studied (Curtis, 1925). In these experiments a number of growing shoots

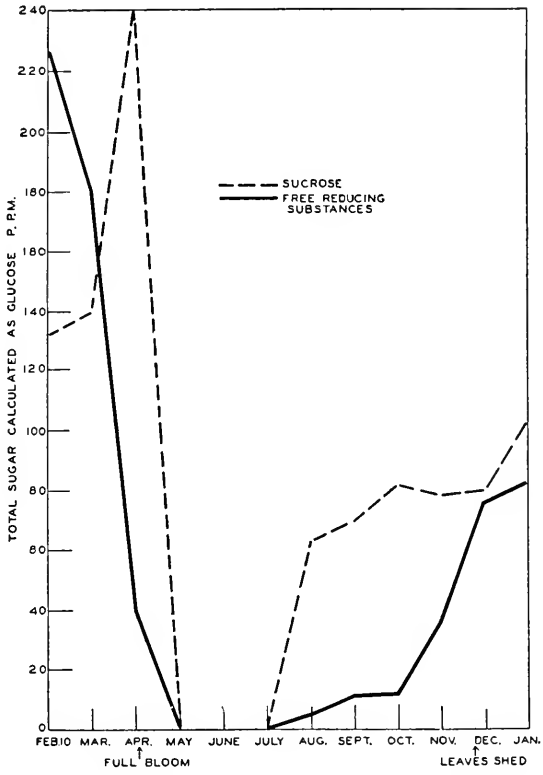

FIG. 107. Seasonal variations in the carbohydrate content of the xylem sap of pear trees. Data of Anderssen (1929).

were first defoliated. Some received no further treatment, thus serving as checks. In others (Fig. 108) a ring of the tissues external to the xylem was removed, and in still others a segment of the xylem was excised, leaving the phloem and cortical tissues intact. Every stem which was cut into was enclosed in a glass cylinder as shown in the figure. This cylinder was filled with water in order to keep the exposed tissue surfaces moist, and in order to supply water to the top of the stems in which the xylem was cut. The outcome of

experiments in which the water jacket was rinsed out once each day with distilled water was essentially the same as in those experiments in which this was not done, indicating that translocation of solutes did not occur through the water.

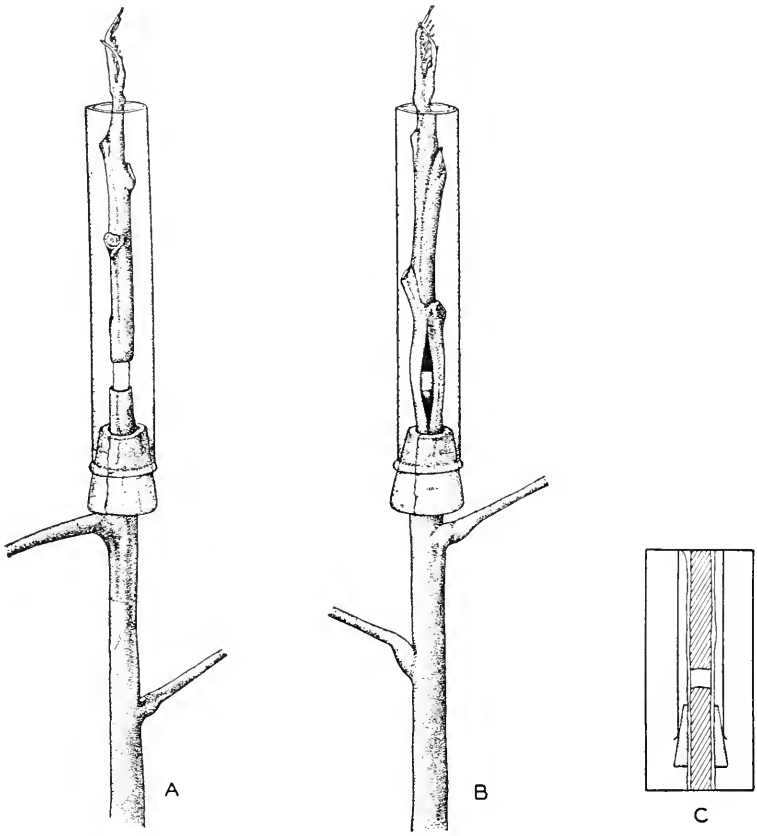

FIG. 108. Diagram to show (*A*) stem with phloem removed, (*B*) with xylem removed, the cut portion of each stem being enclosed in a water jacket, (*C*) sectional view of (*B*). Only the petioles of the leaves are shown. Redrawn from Curtis (1935).

The results of some of these experiments are presented in Table 45. Invariably the stems in which the xylem was cut showed greater elongation than those in which the phloem was cut, indicating that most of the upward translocation of foods occurred through the phloem tissues.

Shoot elongation is a somewhat indirect measure of translocation, but the conclusions drawn from such observations were supported in a number of

experiments by dry weight determinations and analyses for sugar. As shown in Table 45 the dry weight and total sugar content per stem were invariably least in the ringed stems. This was also usually true for the percentage of sugar in terms of fresh weight or dry weight.

TABLE 45—COMPARATIVE EFFECTS OF CUTTING THE XYLEM OR PHLOEM ON GROWTH, DRY WEIGHT, AND SUGAR CONTENT OF DEFOLIATED SHOOTS (DATA OF CURTIS, 1925)

Species	Treatment	Ave. total growth, mm.	Dry weight, per cent of fresh growth	Total sugar per stem, mg.	Sugar, per cent fresh weight	Sugar, per cent dry weight
Mock Orange (*Philadelphus pubescens*) June 13–June 19	Check	63.6	10.8	3.08	0.12	1.12
	Phloem cut	7.8	9.0	0.08	0.03	0.35
	Xylem cut	49.2	10.8	5.32	0.22	2.03
Mock Orange (*Philadelphus pubescens*) June 25–July 1	Check	105.3	13.0	2.10	0.094	0.72
	Phloem cut	19.7	9.4	1.63	0.087	0.93
	Xylem cut	47.4	11.8	4.83	0.231	2.08
Sumac (*Rhus typhina*) June 26–July 1	Check	63.0	22.3	4.17	0.33	1.48
	Phloem cut	15.8	17.2	3.05	0.67	3.89
	Xylem cut	49.5	20.5	3.90	0.42	2.05

In general the evidence seems to warrant the conclusion that the phloem is the principal tissue in which upward translocation of organic solutes occurs, although it seems probable that small quantities of such compounds are, on occasion, translocated in an upward direction through the xylem.

Upward Translocation of Mineral Salts.—For many years it was universally agreed that the upward translocation of mineral salts[2] occurred through the xylem. Current concepts, however, are not nearly so unanimous.

[2] The term "mineral salts" is necessarily employed somewhat loosely. It is used in the immediately following discussion to include nitrogen which may be translocated upwards in either organic or inorganic forms (*cf.* discussion in Chap. XXVI on synthesis of amino acids in roots). Some translocation of other elements, especially sulfur and phosphorus, may also occur in the form of organic compounds since these elements may be converted from inorganic to organic combination at any time after they are absorbed.

Some contemporary authorities contend that the existing evidence supports the thesis that the xylem is the principal channel through which upward transport of such solutes occurs. Others are convinced that the available data indicate that the phloem is the principal tissue concerned. These contradictory views may ultimately be reconciled in the conclusion that both of these tissues serve as important routes of transport.

Formerly the impression was generally prevalent among botanists that mineral salts are carried into the plant along with the water since both are absorbed by the younger parts of roots. As the discussion in Chap XII indicates it is now widely recognized that the absorption of water and mineral salts are largely if not entirely independent processes. This does not necessarily indicate, however, that translocation of water and translocation of mineral salts are also independent processes. It seems entirely possible that salts may pass into the xylem ducts and be translocated upwards in the rising water columns.

Studies of the sap from xylem vessels show that it usually contains at least traces of both organic and inorganic solutes. The previous discussion has indicated that the occurrence of soluble carbohydrates in the xylem sap cannot be accepted as convincing evidence that the xylem is the principal channel in which upward translocation of such compounds takes place. The similar presence of inorganic elements cannot, however, be so heavily discounted. In proportion to the total quantities utilized, the concentration of inorganic constituents in the xylem sap is usually relatively higher than that of organic solutes. Furthermore appreciable concentrations of mineral salts are often present in the sap of vessels at seasons when upward flow of water is occurring at its most rapid rates. At such times the xylem sap contains little or no organic material in solution.

The presence of inorganic constituents in the vessel sap is shown clearly by the work of Anderssen (1929). He obtained samples of tracheal sap from branches of the pear and other species by displacement with gas. The concentration of some of the minerals tested for was found to be distinctly higher in the tracheal sap than in the soil solution (Table 46).

The same investigator studied the seasonal changes in the electrolyte concentration of the tracheal sap of pear branches by determining its specific resistance from month to month during the year. The greater the specific resistance, the less the electrolyte concentration of the sap. As shown by his data (Fig. 109) the maximum concentration of electrolytes occurred shortly after full bloom, following which it slowly and mostly consistently decreased until the minimum was attained in February. In the early spring the trend

TABLE 46—INORGANIC CONSTITUENTS IN THE TRACHEAL SAP OF BARTLETT PEAR AND IN THE SOIL EXTRACT (DATA OF ANDERSSEN, 1929)

Constituent	Soil Extract, Nov. 10		Tracheal Sap	
	18 in. depth, parts per million	36 in. depth, parts per million	Nov. 10, parts per million	May 10, parts per million
Ca	18.0	7.5	16.6	84.7
Mg	7.2	5.4	0.8	23.5
K	140.9	230.0	23.6	59.6
Fe	1.8	1.0	1.0	2.1
SO_4	37.2	30.0	8.3	31.8
Cl	14.0	9.5	3.2	4.5
PO_4	1.0	trace	10.6	25.2
Totals.....	220.1	283.4	64.1	231.4

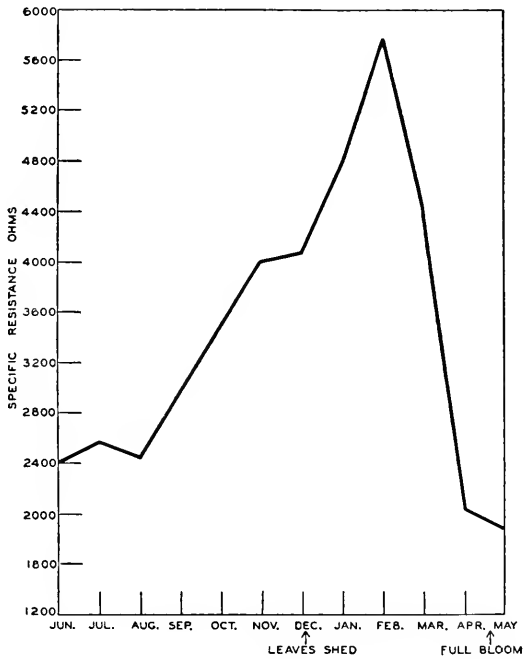

FIG. 109. Seasonal variations in the specific resistance of the xylem sap of pear. Data of Anderssen (1929).

was reversed, and there was a rapid increase in the electrolyte concentration of the sap. During the summer months, when the highest rates of transpiration occur, the electrolyte concentration of the sap was appreciable. These data furnish presumptive evidence that upward translocation of mineral salts occurs in the xylem but do not prove it to be the only or even the main tissue involved in this process.

Evidence indicating that in cotton plants upward translocation of nitrogen occurs principally in the xylem is furnished by the work of Mason, Maskell, and Phillis (1936). These workers used plants which had been grown up to the time of the experiment in sand cultures which were deficient in nitrogen. At the beginning of the experiment the plants were divided into four groups, one of which was used for the initial analysis for nitrogen content. The phloem was then intercepted in the stems of the plants of the second group, and the xylem intercepted in the stems of the plants of the third group. The fourth group was used as a control. The plants were defoliated, supplied with a solution rich in nitrogen and enclosed within bell jars containing wet cotton. After four and eight days analyses for nitrogen were made of the tops and roots from plants of each group. The results (Fig. 110) show clearly that nitrogen moved up almost as effectively in the stems in which the phloem was intercepted as in the controls, while cutting out a section of xylem greatly reduced nitrogen transport. The roots of the plants from all three groups, on the other hand, accumulated approximately equal quantities of nitrogen. The results of this experiment make it seem very probable that most of the translocation of nitrogen in cotton plants occurs through the xylem. One possible criticism of this experiment, however, is that the rather high concentration of nitrogen in the soil solution may have caused a departure from normal in the path of translocation.

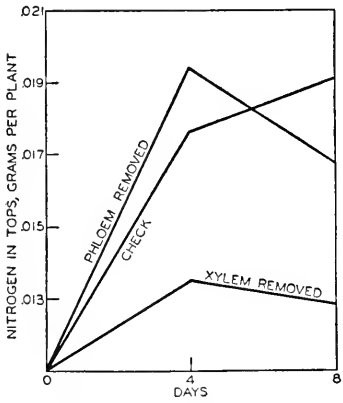

FIG. 110. Effect of intercepting xylem and of intercepting phloem on upward transport of nitrogen in cotton plants. Data of Mason, Maskell, and Phillis (1936).

The results of Clements and Engard (1938) also indicate that at least a part of the mineral salts are translocated upwards through the xylem in a number of woody species. Although ringing the stems decreased the quantity of mineral salts translocated, an effect which was attributed by these workers largely to indirect effects on the xylem as a result of ringing, considerable

upward transport of mineral salts occurred even through stems from which the phloem had been removed.

While there is thus considerable evidence which indicates that upward translocation of mineral salts occurs principally through the xylem, the work of other investigators points to the diametrically opposite conclusion, *i.e.* that the phloem is the main path of mineral salt transport.

The results of a number of experiments by Curtis (1935) support this view. The data from one of these are summarized in Table 47.

TABLE 47—EFFECT OF RINGING ON INCREASE IN NITROGEN AND ASH CONTENT BY LEAVES OF PRIVET (*Ligustrum* SP.) (DATA OF CURTIS, 1935). EACH FIGURE IS THE AVERAGE PER STEM OF 12 SEPARATE DETERMINATIONS

	Check			Ringed			Per cent increase in check in excess of that of ringed
	Aug. 25	Oct 3 and 4	Ave. increase per cent	Aug. 25	Oct. 3 and 4	Ave. increase per cent	
Area of leaves, $dm.^2$..	0.956	1.104	16.6	0.902	1.017	12.9	3.7
Dry weight, g.....	1.130	1.376	22.9	1.033	1.696	66.2	−43.3
Total nitrogen, mg..	16.43	34.51	115.9	14.10	17.38	22.7	93.2
Nitrogen, mg. per $dm.^2$.....	17.07	32.36	82.4	16.14	16.88	6.5	75.9
Nitrogen, mg. per g. dry weight.....	14.41	24.81	67.6	13.90	10.13	−25.5	105.3
Total ash, mg.....	85.4	139.9	61.1	79.8	95.2	20.7	40.4
Ash, mg. per $dm.^2$...	92.0	127.2	38.8	91.9	95.1	3.8	35.0
Ash, mg. per g. dry weight.....	77.8	101.4	30.8	79.8	57.1	−27.2	58.0

This experiment was performed upon privet stems after shoot elongation and xylem formation had ceased for the season. One leaf from each pair was removed at the beginning of the experiment (August 25) from 24 stems. Half of these were ringed so as to remove the phloem and leave the xylem intact; the other half served as checks. The remaining leaves were collected on October 3. Each set of leaves from each stem was analyzed for nitrogen and total ash.

These data seem to indicate that more nitrogen and inorganic solutes were transported through the intact than through the ringed stems, and appear to point definitely to the phloem as the principal route of transport. Since ringing of stems invariably seems to result in a reduction in the transpiration rate the objection might be raised that the mineral salts are being

translocated upwards in the xylem, and that it is the diminution in transpiration rate which accounts for the smaller amounts of translocation through ringed stems. However, for various reasons which cannot be considered in a brief discussion this interpretation seems less probable than the simple assumption that the phloem is the channel of transport.

Moose (1938) has shown that the phloem exudate of several different species contains calcium, magnesium, potassium, lithium, barium, boron, copper, manganese, strontium, and nitrate nitrogen. The presence of such inorganic constituents in the phloem sap is presumptive evidence that at least some translocation of mineral salts occurs through the phloem.

The evidence at present available does not indicate clearly whether translocation of mineral salts occurs predominantly in the xylem, whether it occurs predominantly in the phloem, or whether both of these are important pathways of transport. Even if conduction of mineral salts occurs chiefly in one of these tissues it seems almost certain that at least *some* transport occurs in the other.

Under some conditions the xylem may be the main channel of transport while under others the phloem may serve in this capacity. Some of the conditions which conceivably may have an influence on the route of translocation of mineral salts are the following:

(1) The predominant route of translocation of mineral salts may be different in different species or in herbs as contrasted with woody plants. In herbaceous species such as cotton, for example, most transport of mineral salts may occur in the xylem, while in woody species they may move principally through the phloem.

(2) Some solutes may move principally through the xylem, others through the phloem. Furthermore the same element may move along different routes depending upon the type of chemical combination in which it occurs. Nitrogen in the organic form (amino acids, etc.) may be translocated through the phloem, but nitrates may travel in an upward direction through the xylem (Loomis, 1935). In those species in which amino acid synthesis occurs predominantly in the roots, such as the apple (Chap. XXVI), upward translocation of nitrogen may occur in the phloem, while in those species in which amino acid synthesis occurs principally in the aerial organs, most upward translocation of nitrogen is probably in the form of nitrates and may occur largely in the xylem. The route followed by some of the other elements—especially sulfur and phosphorus—may also depend upon whether they are in organic or inorganic combination. The tissue through which the solute is translocated may also differ with the direction of its movement. As shown in the later discussion even those workers who believe upward translocation

of mineral elements occurs in the xylem agree that outward transport of such compounds from leaves occurs mostly in the phloem.

(3) The concentration of solutes in the medium in which the plant is rooted may affect the path of transport. If a given solute is present in low concentrations most of it may move up through the phloem. With higher concentrations a large proportion of the solute molecules or ions may "leak" past the phloem and be carried up in the xylem. The possibility that inorganic solutes are actually secreted into the xylem ducts (Chap. XVII) must also be considered.

(4) Metabolic conditions within the various organs of the plant, especially the roots, may have an influence on the path of transport. If, for example, the root cells are relatively well stocked with carbohydrates a large proportion of nitrogen absorbed may be utilized in the synthesis of amino acids in the roots, at least in some species. If, on the contrary, the roots are deficient in carbohydrates very little of the nitrogen absorbed will be converted into organic forms in the roots. The previous discussion has indicated that the path of translocation of nitrogen may depend upon the kind of chemical combination in which it exists. Since ringing the stem of a plant below the foliage sooner or later leads to carbohydrate starvation of the roots this factor must be considered in the evaluation of such ringing experiments. It is quite possible that ringing, under some conditions at least, may induce movement of solutes in a tissue in which it normally does not occur.

The results of Gustafson and Darken (1937) support the view that at least some mineral elements are translocated through both the xylem and the phloem. Phosphorus which had been made artificially radioactive was supplied to plants as an 0.5 per cent solution of KH_2PO_4. The presence of such phosphorus in plant tissues can be detected by means of an electroscope. Rooted cuttings of willow, geranium, bryophyllum, and a species of *Sedum* were used as the experimental material. Various types of experiments indicated that the phosphorus ascended the stems of these species in both the xylem and the phloem. As nearly as a quantitative estimate could be made it appeared that these two tissues were about equally effective in conducting phosphorus under the conditions of these experiments.

Export of Solutes Other than Carbohydrates from Leaves and Other Lateral Organs.—Outward translocation of solutes from leaves and other lateral organs is often referred to as "export" of solutes. Leaves not only export carbohydrates, but at times at least, mineral elements in either organic or inorganic combination. According to Mason and Phillis (1937) nitrogen, phosphorus, potassium, sulfur, magnesium, and chlorine all may be exported from leaves, while calcium is practically immobile. Nitrogen, phosphorus,

and sulfur are probably exported in organic combination, while potassium, chlorine, and magnesium apparently move out in inorganic form. Apparently translocation of all of these substances out of the leaf occurs through the phloem. In general those elements which are readily redistributed in plants (Chap. XXV) are the ones which are readily translocated out of leaves.

There is some evidence that a daily variation in the mineral content of leaves may be of regular occurrence. For example, Penston (1935) found the potassium content of mature potato leaves to reach a peak about 3:00 P.M. and to decline during the evening hours. This was apparently also true of other mineral elements. This investigator considers that potassium is probably transported to the leaves in the transpiration stream and is exported from them through the phloem.

Prior to leaf abscission a large proportion of the mineral elements and nitrogen in the foliage of tree species is translocated back into the stems and roots (Deleano, 1936). A similar phenomenon occurs in flowers. Shortly before abscission of the petals of cotton blossoms, nitrogen, magnesium, phosphorus, potassium, chlorine, and magnesium are translocated out of the petals into other parts of the plant (Phillis and Mason, 1936 b).

Mechanism of Translocation of Solutes in the Xylem.—Although at present we cannot estimate with certainty the proportion of the mineral salts translocated upwards through the xylem it is unquestionably true that at least some of the translocation of inorganic compounds occurs through this tissue. Under certain conditions small amounts of organic solutes may also be moved upward in the xylem. Any solutes conducted through the xylem are carried along with the ascending streams of water which are pulled up through the plant according to the mechanism which has already been discussed. In the leaves these solutes move out of the xylem conduits into adjacent living cells. Some solutes may also be lost from the xylem stream by diffusion into living cells of the stem which abut on vessels or tracheids. The rates at which solutes are translocated upwards through the xylem of the stem will correspond with the rates of translocation of water (Chap. XV).

Mechanism of Translocation of Solutes in the Phloem.—It has long been recognized that simple diffusion is inadequate to account for the known rates of translocation through the phloem. This realization has led to the postulation of other theories of the mechanism of transport in this tissue.

1. *The Mass Flow Hypothesis.*—This theory of translocation was first proposed by Münch (1927, 1930) and in modified forms has also been strongly advocated by Crafts (1931, 1932, 1933, 1938). The principles involved in this hypothesis can most easily be clarified by reference to a simple physical system (Fig. 111).

As shown in this figure two differentially permeable membranes, both dipping in water, are connected by a tube to form a closed system. Membrane X is assumed to enclose a stronger solution of sucrose than membrane Y. Water will at first enter both membranes, but the greater turgor pressure developed in X will soon be transmitted throughout the system. This will result in a greater diffusion pressure in the water in Y than in the pure water in which the membranes are immersed. Water will therefore pass out of the membrane Y, and coincidently there will be a flow of *solution* along the tube from X to Y. The mass movement of solution from X to Y will continue until the concentrations of the sugar solutions in both membranes are

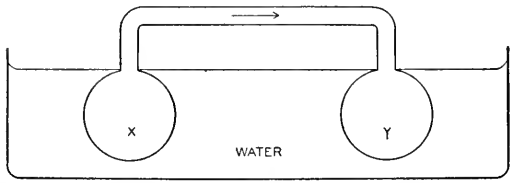

FIG. 111. Diagram of an osmotic system in which mass flow of solution will occur.

equal. At this point the flow of solution in the tube will stop and a dynamic equilibrium will be established between the solution in the closed system and the circumambient water.

If such an apparatus could be set up so that the sugar could be utilized or be converted into an insoluble form as fast as it was translocated into Y, and so that additional sugar could pass into solution in X as rapidly as it moved out of that membrane, flow of solution from X to Y would continue indefinitely.

The Münch hypothesis assumes that a system analogous to that just described accounts for translocation of solutes through the phloem. Fig. 112 (from Crafts, 1931) illustrates diagrammatically how it is supposed to operate as applied to the downward translocation of solutes. Cells L_1, L_2, and L_3 represent the green cells of the leaf and correspond to membrane X in Fig. 111. Similarly R_1, R_2, R_3 represent root cells which are analogous to membrane Y in Fig. 111. The continuous system of phloem connecting leaf and root cells is represented by P. Similarly X represents the xylem and C the cambium. The osmotic pressure of the leaf cells is maintained at a relatively high value as a result of photosynthesis. In the root cells the osmotic pressure is (usually) relatively lower because most of the sugars translocated into them are used in metabolic activities or are converted into insoluble storage forms. Water is supplied continuously to the leaf cells through the xylem.

This hypothesis assumes that the higher turgor pressure of the leaf cells will cause a mass flow of solution downward in the phloem toward the roots. Plasmodesms connecting adjacent cells are supposed to permit mass movement of solution from leaf cell to leaf cell, and from leaf cells into the phloem elements. Movement from one sieve tube to another is supposed to be facilitated by the communicating pores. Some of the solute molecules may be lost to the cambium and other living cells of the stem, but it is assumed that the greater proportion of them are translocated to the roots. The water component of the downward moving solution is supposed to be exuded back into the xylem from the cambium or other receiving cells.

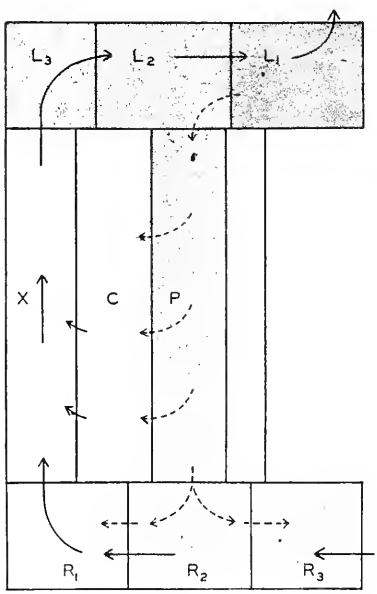

FIG. 112. Diagram to illustrate mechanism of solute translocation according to the Münch hypothesis. Redrawn from Crafts (1931).

This theory will account for translocation through the phloem in only one direction at a time. Transport might occur at times in one direction and at times in the other. If the phloem acts as a single osmotic system, as assumed, flow will occur from the end at which the turgor pressure is higher towards the other. In the spring, for example, if it is postulated that the turgor pressure of the supplying cells in the stem is greater than that of the growing stem tips upward flow would usually occur. Later in the season, as the turgor pressure of the leaf cells increases, a reversal in the direction of flow would be expected.

The principal evidence which has been cited in support of this hypothesis is that sap will often exude rapidly from a cut made into the phloem of a stem (Münch, 1930; Crafts, 1936). The latter investigator has demonstrated that a continuous exudation of phloem sap will occur from stems of squash for a period of at least 24 hours. Because the exudate rapidly coagulates in contact with air, thus plugging the phloem, the flow is maintained only if a fresh cut is made at frequent intervals. Exudation occurs at rates varying from 0.01 to 0.1 cc. per minute. This suggests the occurrence of a mass flow under pressure in the phloem elements of intact stems. It has not been positively shown, however, whether the exudation is occurring from the sieve tubes or other

living cells of the phloem. In some species such as cotton, which have been examined for this phenomenon, it has not been found possible to demonstrate phloem exudation. Some investigators have suggested that such exudations are abnormal flows resulting from cutting open the phloem system, but it seems difficult to apply this interpretation to some of Crafts' results. In one of his experiments the sap exuded in 24 hours was equivalent to the total phloem volume in 189.9 cm. of stem. Furthermore phloem exudation could still be shown in stems which were distinctly wilted, indicating that it is very unlikely that the flow of sap from the phloem could be due to the pressure exerted by adjacent cells as is sometimes suggested.

Although such phloem exudations are undoubtedly very real phenomena, and their occurrence has been demonstrated in many species, it cannot yet be stated with certainty that the actual mechanism of such flows is that postulated in the mass flow theory of translocation.

Curtis (1935) has pointed out some very serious objections to this hypothesis. Several of the more important of these will be summarized.

1. Resistance to the mass flow of solution through a tissue such as the phloem would undoubtedly be very great. The solution must flow through numerous cross walls, and through the cytoplasmic membranes of the sieve tubes. If the solution moves *en masse* through the plasmodesms of the root and leaf cells, as postulated by Münch, this would add greatly to the resistance which must be overcome. Resistance to passage through parenchyma or companion cells would be even greater than in sieve tubes. It seems unlikely that turgor pressures develop in leaves or other supplying tissues of sufficient magnitude to move a solution for any very great distance against the resistance it would encounter in the phloem tissue.

2. The Münch hypothesis requires that the supplying cells have a higher turgor pressure than the receiving cells if there is to be a pressure gradient from the former to the latter. Such a gradient could seldom be maintained unless the *osmotic pressure* of the supplying cells is greater than that of the receiving cells. The investigations of Curtis and Scofield (1933) indicate that this is not always true. According to their results, in the onion, potato, bean, and other species, the osmotic pressures of the receiving tissues are greater than those of the storage tissues at times when translocation is occurring from the latter to the former. This is also true in the sugar beet in which the sugar content of the root cells is much higher than that of the leaf cells or the phloem. This objection to mass flow theory may be met, however, by the assumption that the receiving cells often or generally are capable of accumulating organic solutes against a concentration gradient. On the other hand Pfeiffer (1937) has made an extensive investigation of the osmotic pressures

in a number of tree species, and, with only negligible exceptions, finds them to be distributed in accordance with the requirements of the Münch theory.

3. The mass flow theory will account for translocation in the phloem in only one direction at a time. There are some indications, however, that translocation in the phloem is simultaneously bi-directional. Phillis and Mason (1936a) claim to have demonstrated that organic forms of nitrogen travel up in the phloem of the cotton plant, at the same time that carbohydrates are moving in a downward direction through the same tissue. Palmquist (1938) has apparently demonstrated that the dye fluorescein will move in one direction through the phloem of bean leaves while soluble carbohydrates are moving through the same phloem in the opposite direction.

If bi-directional movement of solutes occurs in the phloem this probably means bi-directional movement in individual sieve tubes. In view of the intimate inter-relationships between phloem elements it seems improbable that solutes could move upward only in certain cells or elements and downward only in others.

2. *The Streaming of Protoplasm Theory.*—De Vries (1885) and other nineteenth century investigators postulated that streaming of the protoplasm in the cells of the phloem might explain the relatively rapid rate of transport of solutes. In recent years this theory has been supported by Curtis (1935). The basic assumption is that rotational streaming of the protoplasm occurs in the sieve tube elements, and that solute molecules, caught in the protoplasmic matrix, are carried by this protoplasmic movement from one end of the element to the other. The molecules are usually assumed to pass from one sieve tube to the next by diffusion, presumably largely through the sieve plates. Diffusion over such short distances can occur very rapidly even if the molecules are moving along a diffusion gradient which is not very steep. Some advocates of this theory have even postulated that streaming protoplasm may be continuous from sieve tube to sieve tube through communicating pores. This theory would account for simultaneous movement of solutes in both upward and downward directions in the same sieve tube.

Although this theory has been rather strongly advocated the positive evidence in its favor is not very convincing. Various types of experiments indicate that the cells of the phloem must be alive in order for translocation to occur. For example, Curtis and Herty (1936) have demonstrated that movement of carbohydrates out of bean leaflets is markedly reduced when the petiole is chilled to a temperature between 0.5 and 4.5° C. At 7-11° C. translocation was faster than at temperatures close to 0° C. and at 17-24° C. it occurred still more rapidly. Curtis (1929) showed that enclosing the stems of bean plants in an atmosphere of nitrogen checked transport, indicating that

oxygen is necessary for the sieve tubes to operate in translocation. Similarly it has been shown that exposure of petioles to narcotics retards translocation of foods out of the leaves. Demonstration that living cells are indispensable for translocation does not necessarily prove, however, that their essential rôle is that assumed by the streaming of protoplasm hypothesis.

Like the mass flow theory the protoplasmic streaming hypothesis also has some serious weaknesses. The more important of these will be summarized.

1. Protoplasmic streaming has not been observed in mature sieve tubes except in water plants, although it can be easily demonstrated in the phloem parenchyma and companion cells. Young sieve tube elements also exhibit active protoplasmic streaming, but all movement of the protoplasm appears to cease as the sieve tubes mature. According to Crafts (1938) the protoplasm of mature sieve tubes is in an inactive, highly permeable condition in which streaming movements have neither been observed nor are to be expected.

2. A second serious weakness in this theory is that the proposed mechanism apparently will not account for calculated rates of translocation, at least as it occurs in some plants. A number of estimates have been made of the rates at which solutes are translocated through the phloem. One of the most recent is Crafts' (1933) calculation of the rate of movement of foods into a potato tuber. All of the carbohydrates which enter a growing potato tuber pass through the slender stolon connecting it with the parent plant. The tubers on which this calculation was based were found to have increased in dry weight at the rate of 0.89 g. per day. Measurements were also made of the cross-sectional area of the various tissues of the stolon. As a result of these measurements it was possible to compute that a 10 per cent solution of sugar must move at a rate of 19 cm. per hour if the total cross-sectional area of the phloem, including the walls, serves as the channel of conduction, or at a rate of 83 cm. an hour if the flow is restricted to the sieve tubes. Some of the other similar calculations indicate even greater rates of movement than this.

The bearing of these data upon the protoplasmic streaming theory is evident from further calculations by the same worker. A probable estimate indicates that only about 5 per cent of the cross-sectional area of the phloem could be occupied by protoplasm flowing in one direction. On the assumption that protoplasm is carrying sugar in the pure state equal to its own volume it must stream at a rate of 56.8 cm. per hour to account for the calculated rate of transport. Since the proportion of sugar actually present in the sieve tubes probably seldom exceeds 25 per cent it is evident that actual rates of streaming must be several times as great as this. Furthermore, in order to account for the observed rates of translocation through the relatively small

apertures of the sieve pores sugar must move in a pure state at a rate of 270 cm. per hour.

The maximum rate of streaming observed by Crafts in the phloem parenchyma of the same species was 1.8 cm. per hour. Protoplasmic streaming at a rate of 47 cm. per hour has been observed in cells of water plants under favorable conditions, and it seems entirely possible that cyclosis in the sieve tubes might occur at rates considerably in excess of the value given above for parenchyma cells. Nevertheless, the rate of streaming seemingly required to account for the measured rates of transport would be considerably greater than this, and it seems improbable that cyclosis occurs at such rates in the sieve tubes.

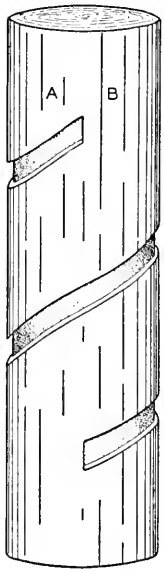

FIG. 113. Diagram to show spiral ringing of a woody stem.

Lateral Translocation of Solutes.—As shown in Chap. XV lateral translocation of water in a tangential direction readily occurs in woody stems. This does not appear to be true of many solutes. In straight-grained trees the sugars from the leaves on one side of a tree are translocated chiefly to the roots directly below them. Similarly if nitrates are added to the soil on one side of a tree, nitrogen is transported principally to the leaves and branches directly above the roots on that side (Auchter, 1923). Lateral transport of at least some kinds of solutes in a tangential direction therefore appears to be slow or negligible in intact stems. Radial transport of solutes along the vascular rays probably occurs much more readily than their lateral movement in a tangential direction.

Experiments of MacDaniels and Curtis (1930) are also of significance in connection with the problem of lateral translocation. When the trunks of apple trees were spirally ringed (Fig. 113) and sodium nitrate added to the soil, it was found that nitrogen was transported principally to the branches above the open end of the spiral (*B*), while those above *A* received relatively little nitrogen. In other words the nitrogen passed spirally around the trunk until it reached the end of the ring, from which point it was transported vertically up the stem. The same results were obtained whether only the phloem was removed, or whether the phloem plus the outermost ring of xylem was cut out. This indicates that the nitrogen was actually being transported through the phloem since cutting this tissue had as much effect as cutting both the phloem and xylem. Since amino acid synthesis in the apple occurs preponderantly in the roots

(Chap. XXVI) translocation of nitrogen in this experiment probably took place in the form of organic nitrogenous compounds.

The results of these experiments indicate that although lateral translocation of nitrogen is relatively slow in intact apple trees that it can be forced by spiral ringing. The effects of spiral ringing upon solute translocation gradually disappear as new conductive tissues develop which are oriented with the axes of the elements parallel to the spiral.

Discussion Questions

1. Why does a girdled tree die eventually but not immediately?
2. Root sprouts develop more often on trees of certain species if they are simply cut down than if they are girdled. Explain.
3. Occasionally a deeply girdled tree has been found to live for many years after ringing. What are some possible explanations?
4. When a woody stem is ringed close to the tip just before bud break the tip sometimes dies. This is often ascribed to injury to the xylem. Is any other explanation possible?
5. How would you determine from how far back in the stem system of a tree the food used by terminal buds in their spring development comes?
6. How would you demonstrate whether translocation of foods away from the leaves occurs more rapidly in the daytime or at night?
7. When a woody stem is girdled dormant buds below the girdle often begin to develop almost immediately. What explanations can you suggest?
8. Why does ringing a stem, no matter how carefully it is done, usually decrease the rate of transpiration from leaves attached above the ring?
9. Pioneers in Indiana made it a rule to girdle trees about August 1. Is there any scientific justification for this date?
10. How could you increase the size of a given apple fruit by girdling? How decrease it?
11. Suppose that certain determinations showed that the nitrogen content of leaves, expressed as a percentage of their fresh weight, is greater in the daytime than at night. Does this necessarily indicate that nitrogen is translocated out of the leaves at night?
12. What experimental procedure would you suggest as a more convincing check of the interpretation given in question 11?
13. Cut stumps of certain species of trees have sometimes been found to remain alive for years after the top of tree has been removed. What are some possible explanations?

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CHAPTER XXIX

RESPIRATION

When seeds germinate in a dark room, the total weight of the developing seedlings will increase for a number of days, but their dry weight will consistently decrease. This can be shown by calculating the dry weight of the seeds at the time of planting from a water content determination of other seeds from the same batch, and determining the dry weight of the resulting seedlings after they have been allowed to develop for several weeks. For example, Boussingault found many years ago that the dry weight of ten pea seedlings allowed to develop in the dark was 1.076 g. while the dry weight of the original seeds was 2.237 g. In other words 1.161 g. or 52 per cent of the dry substance initially present in the seeds disappeared during the course of the experiment. By chemical analysis it can be shown that the loss of dry weight of seedlings growing in the absence of light is due entirely to the disappearance of a portion of the stored foods in the seed. The gain in total weight of such seedlings is due to the absorption of water which occurs during the early stages of germination in quantities far surpassing any loss of dry weight due to the disappearance of foods. The quantity of mineral salts absorbed by young seedlings in the course of a week or two is usually too small to have any appreciable effect upon either their dry or total weight.

If seedlings developing in the dark are enclosed in a chamber which is so constructed that a slow, continuous stream of air can be passed through it, and frequent analyses made of the air, it can be demonstrated that oxygen is continually diffusing into the seedlings and carbon dioxide is continually diffusing out of them.

Furthermore, if such seedlings are enclosed in a calorimeter, and other suitable precautions taken which will be described later, it can also be shown that heat—which is one kind of energy—is continuously escaping from them.

All of these phenomena—disappearance of food resulting in a decrease in dry weight, absorption of oxygen, evolution of carbon dioxide, and liberation of energy—are different external manifestations of the process of *respiration* which occurs, not only in germinating seeds and seedlings, but in almost every living cell.

Aerobic Respiration.—Respiration of the type described in the preceding paragraphs is termed *aerobic respiration,* in order to distinguish it from another less frequent, but not unimportant kind of respiration called *anaerobic respiration,* which also occurs in the higher plants. Aerobic respiration, as the term indicates, refers to respiration which proceeds at the expense of atmospheric oxygen. Anaerobic respiration, on the contrary, occurs in the absence of a supply of atmospheric oxygen. In this chapter only the first of these two processes will be considered.

On the assumption that a hexose is the respiratory substrate the gross chemical aspects of the process are represented by the following equation:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + 673 \text{ kg.-cal.}$$

The value 673 kg.-cal. is based on the assumption that glucose is the hexose oxidized. However the quantity of energy released by the oxidation of other hexose sugars deviates only slightly from this value. This equation is exactly the reverse of the photosynthetic equation and precisely the same quantity of energy is required in the synthesis of one mol of a hexose sugar as is released when one mol of it is oxidized in respiration.

The oxidation of a hexose in plant cells does not take place in a single step as indicated in this convenient summary equation. The possible intermediate steps in the respiratory process will be considered in the next chapter. This equation merely tells us that for the oxidation of one mol of a hexose, six mols of oxygen are required; that six mols each of carbon dioxide and water result from this oxidation, and that 673 kg.-cal. of energy are released. Since equimolar weights of gases occupy the same volume (Avogadro's hypothesis) the volume of oxygen consumed when a hexose is oxidized is equal to the volume of carbon dioxide released.

The water produced as a result of respiration becomes a part of the general mass of water present in the respiring cells. Since it is seldom possible to measure experimentally the quantities of water released in respiration, the conclusion that it is an end product of this process is based largely on theoretical considerations. The water produced in respiration is often termed *metabolic water.*

The gaseous exchanges accompanying respiration were discovered and extensively studied before any especial significance was attached to them. This was true both for plants and animals in both of which oxygen is usually consumed, and carbon dioxide is usually released during respiration. The term respiration has therefore long been used to refer to these externally apparent gaseous exchanges, and is still employed in this sense by most animal

physiologists. As thus employed with reference to the higher animals the term is essentially synonymous with "breathing."

However gaseous exchanges of the usual type are not invariably accompaniments of the process of respiration. Furthermore plants never "breathe" in any fundamentally acceptable sense of the word, frequent popular and semipopular statements to the contrary notwithstanding. Gases pass into or out of plant organs by diffusion through the stomates, lenticels, or directly through the peripheral walls of epidermal cells. Within the plant body gases may be distributed by diffusion through the intercellular spaces or by cell to cell diffusion as solutes. For these reasons plant physiologists use the term respiration primarily to refer to the oxidation of foods in living cells resulting in the release of energy. This is the one basic aspect of the process which is common to virtually all living organisms.

There is considerable evidence that hexose sugars are generally and perhaps invariably the substrate which is oxidized in the cells of higher green plants. When plant cells contain both carbohydrates and fats, the former apparently are consumed first in respiration before any inroads are made upon the accumulated fats. When fatty seeds are allowed to germinate in contact with a sugar solution it has been found that the sugar is oxidized first. Even when fats serve as the respiratory substrate in plants they are apparently first converted into simple sugars (in itself an oxidation process), which are in turn oxidized to carbon dioxide and water. Utilization of proteins in respiration apparently occurs only in tissues which have been depleted of carbohydrates or fats. In starved leaves, for example, proteins are hydrolyzed to amino acids which are first oxidized to asparagine. Under more extreme conditions oxidation of amino acids occurs, resulting in the release of ammonia in the plant tissues (Chap. XXVI). Under such conditions it is believed that the protoplasmic proteins may themselves be hydrolyzed and oxidized.

In most plant organs the rate of respiration is relatively so slow that any heat produced is rapidly dissipated into the environment and thus escapes detection. The evolution of heat during the respiration of certain plant organs can, however, be demonstrated under natural conditions. Temperatures as much as 15° C. in excess of the surrounding atmosphere have been found in the spadices of the skunk cabbage (*Spathyema foetida*), while the temperatures within the spadices of *Arum italicum* have been shown to sometimes exceed atmospheric temperatures by as much as 36° C. This latter, however, must be regarded as an extreme example. Such a self-induced increase in the temperature of a plant organ in itself results in an increase in the rate of respiration and other metabolic processes occurring within that organ.

Heat production during respiration can be demonstrated most easily by

enclosing plant material with a relatively high rate of respiration in a calorimeter. Among such materials are rapidly growing stem tips, opening buds, floral parts (especially during the earlier stages in their development) and germinating seeds. The latter are most frequently used. To conduct the demonstration two Dewar flasks or thermos bottles are partially filled, the one with germinating seeds, the other with an equal mass of germinating seeds which have been killed just before starting the experiment. The calorimeters are then plugged with cotton through which is inserted a thermometer, and the temperature changes noted over a period of time. If the results obtained are to be considered at all critical, the seeds and all parts of each apparatus must be sterilized at the beginning of the determination; otherwise most of the temperature rise observed will be due to the respiration of microorganisms. Such a rise indicates the evolution of heat during the respiration of such organisms, but invalidates the experiment as a demonstration of heat release by the seeds. In general, in a properly set up experiment of this type, the temperature within the mass of living seeds will rise to a value considerably in excess of that recorded for the dead ones. If the dead seeds have been effectively sterilized they will show little or no change in temperature. In such experiments 100 g. of germinating seeds may release heat with sufficient rapidity as to acquire temporarily a temperature as much as 20° C. higher than that of the dead seeds in the check experiment. In the more critical experiments upon heat release by plant tissues the heat evolved is expressed in terms of calories per unit of time (Table 48).

Although the energy released in respiration is generally expressed in terms of heat units (*i.e.* calories or kilogram-calories), not all of the energy is evolved as heat. That portion of the energy released as heat is all lost to the cells in which respiration occurs. It is pure waste as far as the plant is concerned and is roughly analogous to the frictional loss of energy in a machine. In warm-blooded animals, in contradistinction to plants, the heat released in the respiratory process is important in maintaining the body temperature.

The total energy released in respiration can be calculated from determinations of the rate of carbon dioxide evolution. If this is done for seeds in a calorimeter simultaneous determinations can be made of the total energy release and the energy which appears as heat. The results of a series of such determinations are shown in Table 48. It is evident from these figures that the proportion of the total energy released as heat gradually increases during germination, but in no case in this experiment did it exceed 50 per cent of the total.

TABLE 48—COMPARISON OF THE TOTAL ENERGY RELEASE OF ONE KILOGRAM OF WHEAT SEEDLINGS AND OF THE ENERGY DIRECTLY MEASURABLE AS HEAT (DATA OF DOYER, 1915)

Day after beginning of germination	Energy released in respiration as calculated from CO_2 production, kg.-cal. at 25° C.	Energy directly measurable as heat, kg.-cal. at 25° C.
2	2135	363
3	3802	540
4	6277	2938
5	6886	3216
6	8837	4341

Energy becomes manifest in living cells as well as in inorganic systems as chemical energy, heat energy, radiant energy, surface energy, mechanical energy, potential energy, etc. All of the chemical energy released from molecules during respiration represents radiant energy which was previously entrapped in the process of photosynthesis. Upon release this energy may be transformed into any of the kinds listed above.

The present state of our knowledge makes it impossible to say precisely in what manner all of the energy released in respiration which does not appear as heat is utilized in plant cells. A supply of respiratory energy is indispensable in the maintenance of living cells, and at least some of the ways in which this energy is utilized have been recognized. The synthesis of fats, amino acids, and many of the other metabolic products of plant cells requires the energy of respiration. Other energy-requiring processes occurring in plant cells include migration of chromosomes and translocation of other cell constituents during cell division, streaming of the protoplasm, growth of stems in opposition to the pull of gravity, growth of root tips through the soil, maintenance of differences of electrical potential in plant tissues and organs, and the accumulation of ions or molecules by plant cells (Chap. XXIV). Most, if not all of the energy utilized in these processes is made available by the process of respiration.

However, not all plant processes rely upon the energy derived from respiration for their motive power. Transpiration, for example, is essentially a modified evaporation process, and the energy utilized in the vaporization of water mostly comes either directly from the radiant energy of sunlight, or from the heat energy of the surrounding atmosphere.

In addition to its fundamental importance as an energy-releasing process, respiration apparently plays at least one other important rôle in plant metabol-

ism. During the oxidation of foods a number of highly reactive intermediate compounds are produced in plant cells. The chemical nature of some of these substances will be discussed in the next chapter. These highly labile compounds apparently take part in numerous reactions leading to the synthesis of various types of more complex compounds which are essential constituents of the living system of plant cells.

Methods of Measuring Respiration.—Respiration rates are measured in terms of the rate of oxygen consumption, or the rate of carbon dioxide evolution, or both. Rates of carbon dioxide release are more commonly determined than rates of oxygen consumption, since the chemical and physico-chemical methods of detecting changes in the rates of carbon dioxide evolution are easier to work with. Respiration rates are often determined by enclosing the plant in a suitable chamber through which air is allowed to flow at an appropriate rate. The carbon dioxide in the effluent gas stream can be precipitated as $BaCO_3$ by bubbling the gas through a solution of $Ba(OH)_2$. The quantity of this compound formed can be measured either volumetrically (*i.e.* by titration), gravimetrically (by determining the weight of $BaCO_3$ precipitate formed), or by measuring the rate of change of electrical conductivity of the $Ba(OH)_2$ solution. It is also necessary to remove the carbon dioxide from the gas stream before it passes through the plant chamber or else to make a check analysis of its carbon dioxide content. If it is desired to determine carbon dioxide liberation and oxygen consumption simultaneously, the simplest procedure is to collect the gas stream in a reservoir after it has passed through the plant chamber and analyze it for the proportions of these two gases present. If the volume of each of these gases which has passed into the plant chamber is also known the gaseous exchanges of the plant can be computed.

Rates of respiration are expressed in terms of either carbon dioxide evolution or oxygen consumption per unit of time, and are usually calculated on the basis of a unit dry weight of tissue. Obviously accurate determinations of respiration rates of chlorophyllous plant organs can only be obtained if they are enclosed in a respiration chamber which is impervious to light.

Comparative Rates of Respiration.—Rates of respiration as expressed either in terms of oxygen consumption or carbon dioxide liberation vary greatly, depending upon the plant organ or tissue. Since the seat of respiration lies in the protoplasm a correlation often exists between the proportion of protoplasm present in a tissue and the intensity of the respiration process in that tissue. As a general rule respiration rates are found to be greatest in meristematic tissues such as growing root or stem tips or the embryos of germinating seeds. It is precisely in such tissues that the proportion of protoplasm is

greatest in relation to the total dry weight of the tissue. In mature tissues, such as photosynthetically active leaves, a larger proportion of the dry weight of the tissue mass is composed of inert cell wall materials, hence the respiration rates of such tissues, expressed in the usual terms, are almost invariably less than those of meristems under comparable conditions. Senescent tissues, such as yellowing leaves or ripe fruits in which the proportion of protoplasm to dry weight is still smaller, generally have lower rates of respiration than the same tissues had when in a metabolically active condition. The lowest rates of respiration are found in dormant seeds and spores, a marked increase in the rate of respiration being one of the striking physiological aspects of germination. The relatively slow rate of respiration in such structures is not, however, due primarily to a low proportionate amount of protoplasm, but to other factors, among which deficient hydration of the tissues is one of the most important.

Representative rates of respiration for a number of plant organs are listed in Table 49. Even for the plant parts tabulated these rates are to be regarded as only approximations, since the rate for any one plant organ or tissue is subject to marked fluctuations due to the influence of various internal and external factors.

TABLE 49—RESPIRATION RATES OF VARIOUS PLANT TISSUES IN TERMS OF VOLUME OF OXYGEN ABSORBED OR VOLUME OF CARBON DIOXIDE RELEASED IN 24 HOURS PER GRAM OF DRY WEIGHT (FROM DATA COMPILED BY KOSTYCHEV, 1927)

Plant	Organ	Temperature	Respiration Rate
Wheat (*Triticum sativum*).....	Young roots	15–18° C.	67.9 cc. O_2 absorbed
Red Clover (*Phleum pratense*)....	Leaves	20–21°	27.2 " " "
Rice (*Oryza sativa*).....	Young roots	14–17°	44.4 " " "
Mint (*Mentha aquatica*).....	Roots	18–19°	37.2 " " "
Lilac (*Syringa vulgaris*).....	Leaf buds	15°	35.0 cc. CO_2 liberated
Linden (*Tilia europea*).....	Leaf buds	.....	66.0 " " "
Lettuce (*Lactuca sativa*).....	Germ. seeds	16°	82.5 " " "
Poppy (*Papaver somniferum*).....	Germ. seeds	16°	122.0 " " "
Mold (*Aspergillus niger*).....	Mycelium	.....	1800 " " "

Kidd, West, and Briggs (1921) have studied the rate of respiration of various organs of the sunflower plant as well as of the entire aerial portion of this plant at different stages of development (Table 50). This is one of the most comprehensive studies which has ever been made on respiration rates in any one species. The principal fact elucidated by this work is that the respiration rate of not only the entire plant, but also the individual organs

such as leaves, stems, and stem tips decreases consistently with increasing age. The rate of respiration of the stem apices is consistently higher than that of the leaves, which in turn exhibit a consistently higher rate than the stems.

TABLE 50—CHANGES IN THE RATE OF RESPIRATION OF SUNFLOWER PLANTS WITH AGE (DATA OF KIDD, WEST, AND BRIGGS, 1921)

Days after germination	Number of plants used	Dry weight of a single plant (g.)	Rate of respiration (mg. CO_2 per g. dry weight per hr.)			
			Entire plant	Stem	Leaves	Stem apex
1	30	0.0225	2.90			
2	25	0.0223	3.00			
4	25	0.0242	3.00			
13	10	0.1009	2.80			
22	8	0.630	3.00			
29	2	4.065	2.30			
36	1	12.85	1.21	0.81	1.56	
43	1	22.05	1.03	0.69	1.38	
50	1	45.15	0.94	0.46	1.52	2.56
59	1	93.20	0.66	0.33	1.32	1.78
64	1	98.30	0.71	0.34	1.24	....
89	1	294.7	0.48	0.31	0.90	1.13*
99	1	377.4	0.37	0.25	0.45	0.89
112	1	818.3	0.26	0.098	0.375	0.75
136	1	419.5	0.39	0.081	0.44	0.96

* From this date the stem apex was the inflorescence only.

The Compensation Point.—In the leaves or other chlorophyllous tissues the rate of photosynthesis usually exceeds the rate of respiration during the daylight hours. The carbon dioxide produced in respiration is re-utilized by the cells in photosynthesis, but since the latter process is occurring more rapidly than the former, additional carbon dioxide is continuously diffusing into the plant from the outside environment. Similarly photosynthesis produces more oxygen than is used in respiration, the surplus diffusing out of the plant. Hence during the daylight hours, as long as conditions favorable for photosynthesis prevail, there is a net movement of carbon dioxide into the green parts of plants, and a net loss of oxygen from them. Under such conditions the occurrence of the gaseous exchanges accompanying respiration in leaves is completely masked.

At night or in the dark the reverse condition obtains, oxygen diffusing into the green parts of a plant and carbon dioxide diffusing out of them. Similar gaseous exchanges are characteristic of the non-green organs of a plant, whether in the light or in the dark. The magnitude of the gaseous

exchanges occurring between a green plant organ and its environment in the absence of light are usually less than those which generally take place—but in the opposite direction—in its presence.

Since at low intensities light is the limiting factor in photosynthesis it is evident there should be a certain light intensity at which the rate of photosynthesis and the rate of respiration in a leaf or other chlorophyllous organ are exactly equal. At this light intensity, often called the *compensation point,* the volume of carbon dioxide being released in respiration is exactly equal to the volume being consumed in photosynthesis, while the converse is true for oxygen. The light intensity corresponding to the compensation point varies greatly with different species of plants. The compensation point for any one species is also influenced by various environmental factors, especially temperature, and is markedly affected by the conditions to which the leaves or other photosynthetic organs have been exposed during their development.

Burns (1923) studied what he termed the "minimum light requirement" of a number of species of forest trees. This was done by enclosing the tops of young potted trees under a bell jar and sealing off the soil surface. He then determined, for each species, the light intensity at which no change occurred in the volume of carbon dioxide in the bell jar during a three hour exposure, which would represent a condition under which the rates of respiration and photosynthesis are equal (Table 51). This light intensity would therefore represent essentially the compensation point for the aerial organs of the tree considered as a unit.

TABLE 51—MINIMUM LIGHT REQUIREMENT (LIGHT INTENSITY AT WHICH PHOTOSYNTHESIS EQUALS RESPIRATION) OF FOREST TREE SPECIES (DATA OF BURNS, 1923)

Species		Percentage of full sunlight
Pinus ponderosa	Yellow Pine	17.0
Pinus sylvestris	Scotch Pine	15.9
Thuja occidentalis	White Cedar	10.3
Larix laricina	Tamarack	9.8
Pseudotsuga mucronata	Douglas Fir	7.6
Pinus murrayana	Lodgepole Pine	7.6
Quercus borealis	Red Oak	7.4
Celtis occidentalis	Hackberry	6.4
Picea engelmannii	Englemann Spruce	5.9
Pinus strobus	White Pine	5.8
Picea excelsa	Norway Spruce	4.9
Tsuga canadensis	Hemlock	4.7
Fagus grandifolia	Beech	4.2
Acer saccharum	Sugar Maple	1.9

As indicated by the results shown in this table the minimum light requirement of species which ecological observations have shown can exist only in well lighted habitats, such as some of the pines, is considerably in excess of the requirement for such shade tolerant species as hemlock, beech, and sugar maple. The maintenance of a species at the light intensity of the compensation point will not, however, permit its survival under natural conditions. In the first place light is available in the natural habitats of plants for only part of each day; hence there is no photosynthesis which compensates for night respiration. In the second place, if, as is the usual practice, the compensation point is measured only for the leaves or aerial organs of the plant, no allowance is made for respiration of the roots or other non-green organs. Furthermore, no plant can survive indefinitely without the consumption of some food in assimilation, and at the compensation point no food would be available which could be used in this process. Hence the actual minimum light intensities at which these species could survive in nature would necessarily be somewhat greater than those indicated in Table 51. However, the *order* of their minimum light requirements under natural conditions probably would not differ greatly from that found in this investigation.

The Respiratory Ratio.—The ratio of the volume of CO_2 released to the volume of O_2 absorbed in the respiratory process is termed the *respiratory ratio* or *quotient*. When complete oxidation of a hexose sugar occurs, as already pointed out:

$$\frac{CO_2}{O_2} = 1$$

The respiratory ratio for any plant or plant part can be determined by making parallel measurements of the rates of carbon dioxide release and oxygen consumption.

The respiratory ratio of germinating seeds in which the accumulated foods are principally in the form of carbohydrates is invariably found to be approximately one as long as oxygen has free access to such seeds. This is true, for example, of the germinating grains of practically all of the cereals (wheat, maize, oats, etc.). Similarly the respiratory ratios for the leaves of many species of plants have been found to be in the neighborhood of one (Table 52) and flowers usually have a respiratory ratio of approximately one (Pringsheim, 1935).

Unlike the photosynthetic ratio, the respiratory ratio of plant tissues is by no means always equal to unity, although most commonly this value is approximated. The proportion of the volume of carbon dioxide evolved to the volume of oxygen absorbed may vary greatly from a unit value, depend-

TABLE 52—RESPIRATORY RATIOS OF THE LEAVES OF VARIOUS SPECIES
(DATA OF MAQUENNE AND DEMOUSSY, 1913)

Begonia	1.11	Pea	1.07
Castor Bean	1.03	Pear	1.10
Chrysanthemum	1.02	Privet	1.03
Corn (maize)	1.07	Rose	1.02
Grape	1.01	Tobacco	1.03
Lilac	1.07	Wheat	1.03

ing upon the respiratory substrate, the completeness of the oxidation, and other conditions. Many studies have been made of the respiratory quotient of various plant organs. Most such investigations have dealt with germinating seeds. The principal internal conditions under which it has been found that the respiratory ratio of the higher green plants deviates from one are as follows:

1. *Respiration of Compounds Which Are Relatively Poor in Oxygen as Compared with the Hexoses.*—The proportion of oxygen to carbon is invariably less in fats than in carbohydrates. Many studies of the respiratory ratio of seeds in which the stored foods are mostly in the form of oils have shown that the respiratory ratio of such seeds is always less than one. The following summary equation represents the complete oxidation of tri-palmitin, a representative fat:

$$C_{51}H_{98}O_6 + 72.5\ O_2 \rightarrow 51\ CO_2 + 49\ H_2O + 7590 \text{ kg.-cal. (approx.)}$$

The theoretical respiratory quotient for this compound is therefore $\frac{51}{72.5} = 0.70$.

Respiratory ratios of less than one would result when fats are oxidized whether they are used directly as the substrate, or whether, as most investigators believe, they are first converted into simple sugars which in turn serve as the respiratory substrate. If fats are oxidized directly, it is evident from the above equation that a greater volume of oxygen will be required than the volume of carbon dioxide which will be released. On the other hand, if the fats are first converted into simple sugars, which then serve as the respiratory substrate, a considerable volume of oxygen will be utilized in accomplishing this transformation, since conversion of fats to sugars is an oxidation process. Transformation of fats to sugars does not result in the release of carbon dioxide. This gas would be released only upon oxidation of the simple carbohydrates. Hence the summation effect of these two processes,

if occurring simultaneously, would be the absorption of oxygen in a volume in excess of the carbon dioxide evolved and the resulting respiratory ratio would be less than one.

Similarly oxidation of the hydrolytic products of the proteins results in a respiratory ratio of less than one since the proportion of oxygen to carbon in such compounds is less than in carbohydrates.

2. *Respiration of Compounds Which Are Relatively Rich in Oxygen as Compared with Hexoses.*—In some species of plants, particularly those of the succulent habit of growth, organic acids are often oxidized. Such compounds are relatively rich in oxygen as compared with carbohydrates. The equations for the complete oxidation of oxalic and malic, two of the common plant organic acids are as follows:

$$2\ \begin{matrix}\mathrm{COOH}\\ |\\ \mathrm{COOH}\end{matrix} + O_2 \rightarrow 4\ CO_2 + 2\ H_2O + 60.2\ \text{kg.-cal.}$$

Oxalic acid

$$\begin{matrix}\mathrm{COOH}\\ |\\ \mathrm{CHOH}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{matrix} + 3\ O_2 \rightarrow 4\ CO_2 + 3\ H_2O + 320.1\ \text{kg.-cal.}$$

Malic acid

The theoretical respiratory ratio for oxalic acid is therefore 4, for malic acid $\frac{4}{3}$ or 1.33. For tartaric acid, another organic acid which occurs in plants, the respiratory ratio is 1.6. Oxidation of any compound of this type results in respiratory ratios in excess of one.

3. *Occurrence of Anaerobic Respiration.*—In this process, which may occur in the higher green plants under certain conditions, carbon dioxide is released without any corresponding absorption of oxygen. Sometimes when the oxygen supply is deficient, both aerobic and anaerobic respiration occur simultaneously in a plant tissue. Some cells may be respiring anaerobically, while others are carrying on aerobic respiration. In the early stages of germination of seeds in which the seed coats are relatively impermeable to oxygen a limited amount of aerobic respiration may be carried on, accompanied by a larger proportion of anaerobic respiration. Such a phenomenon may be observed, for example, in pea seeds. Under such conditions the volume of carbon dioxide evolved may be very large in proportion to the volume of oxygen absorbed and the respiratory ratio is much greater than one. As

soon as the seed coat is ruptured, thus permitting free access of oxygen to the developing embryo, anaerobic respiration ceases.

4. *Occurrence of Other Processes Resulting in the Release or Consumption of Oxygen.*—The respiratory ratio is measured in terms of the rate of absorption of O_2 and the rate of evolution of CO_2. Respiration is not, however, the only process occurring in plant cells which results in the utilization or liberation of oxygen. The simultaneous occurrence in plant cells of other oxygen releasing or consuming processes and respiration will influence the *apparent* respiratory ratio, and there is no critical method of distinguishing between such apparent ratios and the true ratio.

For example, as seeds which store fat mature, simple carbohydrates are converted into fats. This process involves the elimination of oxygen, since the molecules of fats contain much less oxygen in proportion to the carbon and hydrogen present than do the molecules of sugars. Such a release of oxygen during fat synthesis furnishes an internal supply of oxygen which can be used in the process of respiration. Hence the volume of oxygen absorbed by the seeds from the exterior atmosphere during this period will be less than the volume of carbon dioxide released, and the apparent respiratory ratio would theoretically be greater than unity. This expectation has been confirmed for the seeds of a number of species which store fats. For example, it has been found that the apparent respiratory ratio of maturing flax seeds is about 1.22.

Essentially the opposite situation prevails during the germination of fatty seeds in which very low respiratory quotients sometimes prevail after several days. Murlin (1934), for example, found values for the respiratory ratio of germinating castor bean seeds as low as about 0.3 and explained this finding on the assumption that transformation of fat to sugar—an oxygen-consuming process—is proceeding much more rapidly than oxidation of either fat or sugar.

In species of the *Crassulaceae* some of the sugar present is often incompletely oxidized to malic acid:

$$2\ C_6H_{12}O_6 + 3\ O_2 \rightarrow 3\ C_4H_6O_5 + 3\ H_2O + 386\ \text{kg.-cal.}$$

Other organic acids are formed in succulent species as a result of similar incomplete oxidations. The synthesis of such compounds requires absorption of oxygen for which there is no corresponding evolution of carbon dioxide. Such metabolic conditions will obviously result in an apparent respiratory ratio of less than one.

In addition to the various internal conditions which influence the value of the respiratory quotient, its magnitude may also vary with certain factors

of external origin. Increase in temperature causes an increase in the respiratory quotient of apple seeds (Harrington, 1923). Internal conditions are often such that the absorption of oxygen and liberation of carbon dioxide are not equally affected by changes in temperature, hence the apparent respiratory ratio may shift with a change in temperature. The magnitude of the respiratory ratio is also sometimes influenced by other environmental factors such as the oxygen or carbon dioxide concentration of the atmosphere (Table 54).

The principal justification for study of the respiratory quotients of plants is that the results of such investigations afford important clues regarding the nature of the respiratory process itself. Certain conclusions regarding the type of substrate being oxidized, transformations in the foods present in cells, and even the chemical mechanism of respiration can often be drawn from the results of determinations of respiratory ratios. Some caution is always necessary, however, in interpreting the results of such experiments. It is probable that a number of different processes are often occurring simultaneously in plant cells, each of which will influence the magnitude of the respiratory ratio. Several types of substrates may be undergoing oxidation concurrently, or other oxygen consuming or releasing processes may be in progress in addition to the direct oxidation of foods. If one of these several reactions is strongly predominant, it will in the main determine the value of the respiratory ratio, and the quotient will be a valid indicator of the nature of the respiratory process. If, on the contrary, several different processes are proceeding at approximately equal rates, their net influence on the gaseous exchanges of the tissue may be such that the apparent respiratory ratio will bear no relation whatever to any one of the processes, and may even lead to entirely erroneous inferences.

Factors Affecting the Rate of Aerobic Respiration.—A number of factors, some internal, others external, are definitely known to influence the rate of respiration of plant cells. These will be discussed under appropriate headings.

1. *Protoplasmic Conditions.*—Young, meristematic tissues, which are relatively rich in protoplasm, usually have higher rates of respiration than older tissues in which the proportion of cell walls is greater. Not only is the gross amount of protoplasm present a factor, but various internal conditions in that protoplasm also influence the respiration rate. In view of our incomplete knowledge of the physico-chemical structure and dynamics of the protoplasm the influence of only a few of these protoplasmic factors can be recognized at the present time. One of these is the degree of hydration of the protoplasm. The effect of this factor will be discussed later under a separate heading.

The quantity of respiratory enzymes present in the protoplasm undoubtedly is also a factor which affects the rate of respiration.

2. *Temperature.*—As is true of most other biological processes temperature effects upon the rate of respiration are rather complex. In general, within certain limits, increase in temperature results in an increase in the respiration rate. As in photosynthesis and in enzymatic reactions a definite time factor effect is often evident. The general principles regarding the effect of temperature upon respiration rates are illustrated by the data shown graphically in Fig. 114. In this experiment pea seedlings were used as the experimental

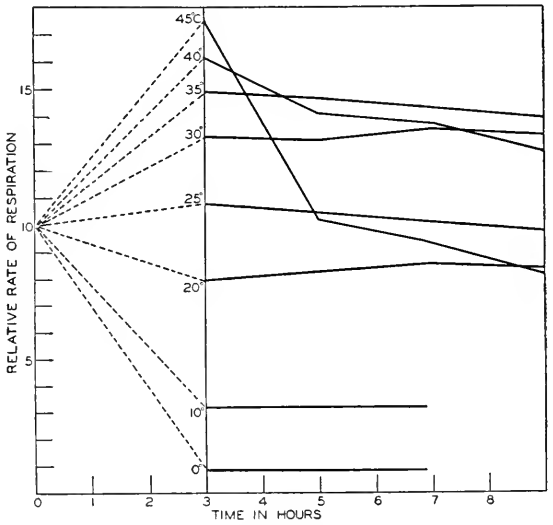

FIG. 114. Relation between time, temperature, and rate of respiration of pea seedlings. Dotted lines represent period during which temperature of seedlings was changed from 25° C. to indicated temperatures. Data of Fernandes (1923).

material. In the temperature range between 0° C. and 45° C. increase in temperature resulted in an increase in the initial rate of respiration. At temperatures above approximately 30° C. the rate of respiration showed a decrease with time, which became more marked the higher the temperature.

For the pea seedlings used in this experiment the optimum temperature would appear to be about 30° C., as this is approximately the maximum temperature at which there is a maintained rate of respiration. The optimum temperature for respiration, considered in this sense, is not the same for all plant tissues. For some it is clearly higher than the value obtained in this experiment with pea seedlings and for others it is lower.

The exact nature of the "time factor" which becomes increasingly effective

in causing reduction in the rate of respiration with rise in temperature is unknown. One of the seemingly more probable explanations is that this effect is due to a progressively more pronounced inactivation of enzymes with increase in temperature. Other possibilities are: (1) oxygen may not gain access to the cells fast enough at higher temperatures to permit maintenance of the respiration rate, (2) carbon dioxide may accumulate in the cells in such concentrations at higher temperatures as to check the rate of respiration, and (3) the supply of oxidizable foods may be inadequate to maintain high rates of respiration.

As the temperature is decreased below 0° C. the rate of respiration gradually diminishes until it becomes imperceptible. Measurable rates of respiration have been recorded, however, in some plant tissues at temperatures as low as −20° C.

The temperature coefficient of respiration, within the temperature range 0° C. to 30° C., appears to be about 2.0 to 2.5.

The temperature to which a plant organ is exposed sometimes has important indirect effects on the rate of respiration. When the temperature of a potato tuber is lowered from a few degrees above to about 0° C. the respiration rate *increases*. According to Hopkins (1924) this is due to the effect of low temperatures in causing a shift in the starch-sugar equilibrium towards the sugar side (Chap. XXII). Increase in the quantity of respiratory substrate in plant cells results in an increase in the rate of respiration whenever it is the limiting factor, a condition which apparently obtains in potato tubers under these conditions. Similar indirect effects of temperature upon the rate of respiration are probably of frequent occurrence in other plant tissues.

3. *Food.*—As a general rule increase in the soluble food content of plant cells results in an increase in the respiration rate up to a certain point at which some other factor becomes limiting. One example of the effect of the concentration of foods in cells upon the rate of respiration has just been described in the previous section. The effect of this factor on respiration rates can also be demonstrated in etiolated leaves. For example, Palladin (1893) found that 100 g. of carbohydrate deficient etiolated bean leaves released an average of 89.6 mg. of carbon dioxide per hour at room temperature. After floating the same leaves upon a sucrose solution for two days, during which considerable absorption of sugar occurred, the average rate of carbon dioxide release was increased to 148.8 mg. per hour.

4. *Oxygen Concentration of the Atmosphere.*—The effects of various oxygen concentrations on the rate of respiration in young wheat seedlings is shown in Table 53.

TABLE 53—RATE OF CARBON DIOXIDE PRODUCTION IN MILLIGRAMS BY 100 YOUNG WHEAT SEEDLINGS AT 25° C. (DATA OF MACK, 1930)

Test interval	Per cent of oxygen in the atmosphere											
	0.6	3.1	6.3	9.8	16.0	20.0	30.0	50.0	75.0	90.0	95.0	98.3
3rd–6th hr. inc.	0.74	0.88	0.93	**1.02**	0.97	0.95	1.05	1.20	1.38	**1.50**	1.29	1.23
7th–12th hr. inc.	0.79	1.00	1.14	**1.37**	1.20	1.16	1.24	1.44	1.81	**2.04**	1.74	1.80
13th–24th hr. inc.	0.93	1.52	1.81	**1.95**	1.74	1.46	1.67	1.93	2.80	**3.32**	3.16	3.18
25th–36th hr. inc.	1.03	1.85	2.06	**2.31**	2.21	1.90	2.00	2.75	3.99	4.56	**5.14**	4.65
37th–48th hr. inc.	1.23	2.14	2.30	2.93	**2.97**	2.45	2.75	3.87	4.94	5.66	**6.30**	5.72

As shown in this table the rate of respiration increased with increase in oxygen concentration up to a first maximum at 9.8 or 16 per cent. Further increase in the partial pressure of oxygen resulted in a fall to a minimum value which was attained at 20 per cent, while still further increase in oxygen concentration resulted in a rise in the rate of respiration until a second maximum was attained at 90 or 95 per cent. Similar results were obtained at other temperatures. The cause of the first maximum of respiration is not clear, and perhaps this would not be shown by all species, or under all experimental conditions. Some anaerobic respiration may have occurred at the lowest concentrations of oxygen employed. In general these data support the results of several other investigators which indicate that the oxygen concentration can deviate considerably from that normally present in the atmosphere without greatly influencing the rate of respiration.

5. *Carbon Dioxide Concentration of the Atmosphere.*—The higher the carbon dioxide concentration, the lower the rate of respiration of some plant tissues. In a study of white mustard seedlings it was shown that the rate of respiration decreased with increase in carbon dioxide concentration (Table 54). This effect was shown whether respiration was measured in terms of

TABLE 54—EFFECT OF CARBON DIOXIDE CONCENTRATION UPON THE RATE OF RESPIRATION OF GERMINATING WHITE MUSTARD SEEDS. INITIAL CONCENTRATION OF O_2 IN EACH EXPERIMENT 20 PER CENT. DURATION OF EXPERIMENTS, 14 HOURS. (DATA OF KIDD, 1915)

	Percentage of CO_2 initially present					
	0	10	20	30	40	80
CO_2 evolved (cc.)	58	48	38	33	26	17
O_2 absorbed (cc.)	71	57	49	45	38	32
Respiratory ratio	0.82	0.84	0.77	0.73	0.69	0.53

carbon dioxide release or oxygen absorption. The decreasing effect on the rate of carbon dioxide release was more marked than on the rate of absorption of oxygen. Hence the higher the carbon dioxide concentration of the atmosphere, the lower the respiratory ratio.

The rate of respiration of some plant tissues, on the contrary, is increased when they are exposed to relatively high concentrations of carbon dioxide. For example, Thornton (1933) studied the rate of respiration of potato tubers at 25° C. in various concentrations of carbon dioxide. The initial concentration of oxygen in the atmosphere was 20 per cent in all determinations. Exposure of the tubers to concentrations of carbon dioxide in excess of about 20 per cent for periods of greater than 20-24 hours resulted in a marked increase in respiration rate as measured in terms of oxygen consumption. In 60 per cent carbon dioxide the rate of respiration sometimes exceeded that of the controls by more than 200 per cent. Shorter periods of exposure resulted in a decreasing rather than increasing effect upon respiration. High concentrations of carbon dioxide similarly resulted in an increase in the rate of respiration of a number of other storage tissues. With asparagus shoots and shelled lima beans, on the other hand, high concentrations of carbon dioxide resulted in a reduction in respiration rate as found by Kidd for germinating mustard seeds. Evidently the effect of carbon dioxide upon the rate of respiration is influenced not only by its concentration, but also depends upon the kind of tissue and the period of exposure.

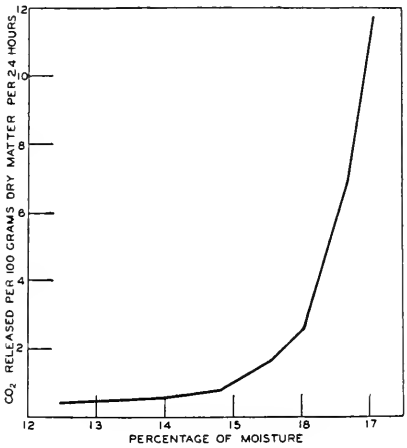

FIG. 115. Relation between water content of wheat seeds and rate of respiration. Data of Bailey and Gurjar (1918).

6. *Hydration of the Tissues.*—As is true of a number of other processes the effect of the hydration of the tissues upon the rate of respiration can best be observed in germinating seeds. The influence of the moisture content of wheat seeds upon their rate of respiration is indicated graphically in Fig. 115, which is self-explanatory.

Similar results have been obtained for tissues of some of the more extreme xerophytes, which can be desiccated to an air dry condition without destroying their viability. As the water content of such tissues is increased, often no

great effect upon the rate of respiration is observed until a certain water content (which varies according to the tissue) is attained, after which the respiration rate increases rapidly.

Minor variations in the water content of well hydrated plant cells do not appear to have any very great influence upon the rate of respiration. Walter (1929) found that a reduction in the hydration of the cells of *Elodea* such as was induced by immersing them in a weight molar sucrose solution had no appreciable effect upon the rate of respiration, although photosynthesis ceased entirely under such conditions.

7. *Light.*—As far as available evidence goes light has no direct effect upon the rate of respiration. It does, however, exert certain indirect effects. In chlorophyllous organs, light may affect the rate of respiration because of its influence upon the supply of respiratory substrate resulting from photosynthesis. Plant organs exposed to direct illumination almost invariably have a temperature in excess of that of similar organs not so exposed. The heating effect of light is one of its important indirect effects upon respiration.

8. *Injury.*—Wounding of plant tissues almost invariably results in a temporarily increased rate of respiration. If a potato tuber is cut in half, for example, the loss of carbon dioxide from the two halves will be considerably greater than from the intact tuber. Similar results have been observed for many other plant tissues. The increased respiratory activity of wounded or otherwise injured plant tissues gradually rises to a maximum which is generally attained within a day or two, after which a diminution in rate sets in until approximately the rate which prevailed in the uninjured tissues is re-established.

Hopkins (1927) and others have shown that this increased respiration of potato tubers following wounding is correlated with an increase in the sugar content of the tuber. This increase, which amounted in Hopkins' experiments to from 53 to 68 per cent of the sugar originally present, occurs gradually, the maximum not being attained until several hours after the injury. The increase in sugar content is greater in the cells close to the cut surface than in those which are more remote from it. This increase in the quantity of the respiratory substrate is apparently an important factor in accounting for the increased loss of carbon dioxide by potato tubers following wounding, and probably of many other tissues as well.

9. *Various Chemical Substances.*—Many investigations of the specific influence of various chemical compounds upon the rate of respiration have been made. Studies of the effects of various toxic organic substances such as chloroform, ether, acetone, ethyl alcohol, formaldehyde, morphine, bromine, quinine, etc. have been especially extensive. As a specific example of the

effect of such substances we will consider the influence of chloroform on the rate of respiration in the leaves of the cherry laurel (*Prunus laurocerasus*) as shown by the work of Irving (1911). Small doses caused an increase in the rate of respiration which persisted as long as the leaf was maintained in contact with the chloroform. Somewhat larger doses resulted in a temporary increase in respiration rate, which was followed by a decrease to much below the initial rate. The greater the concentration of the chloroform, the more rapid this decrease. Strong doses of this reagent resulted in a rapid decrease in the rate of respiration without the occurrence of any preliminary increase. In general the effect of other compounds of this type upon respiration is very similar to the effect of chloroform. It is noteworthy that the rate of photosynthesis is markedly reduced by concentrations of chloroform so minute that they have no detectable effect upon the rate of respiration.

The Photosynthetic-Respiratory Ratio.—No plant can survive indefinitely under conditions which permit maintenance of a rate of respiration in excess of the rate of photosynthesis. Although plants subjected to such conditions can "coast" for a while at the expense of previously stored foods, eventually they will starve to death. In other words, if a plant is to survive indefinitely its photosynthetic-respiratory ratio (P/R ratio) must exceed unity. The magnitude of the P/R ratio in any plant will be affected by any factor which influences either or both of the processes of photosynthesis and respiration. Of the factors which affect this ratio, temperature is one of the more important and the following discussion will be restricted to a consideration of its effects.

In many and perhaps all species of plants the optimum temperature for respiration is higher than the optimum for photosynthesis. For example, in the potato plant the optimum temperature for photosynthesis, under conditions of full daylight and normal atmospheric concentration of carbon dioxide, is about 20° C. The optimum temperature for respiration of the leaves of the potato plant, on the other hand, is apparently about 35° C. (Lundegårdh, 1931). With increase in temperature above 20° C. there is little further rise in the rate of photosynthesis in this species or there may be an actual decrease. The rate of respiration, on the contrary, continues to rise with increase in temperature up to about 35° C. Hence the higher the temperature above the photosynthetic optimum the greater the proportion of the photosynthate which will be consumed in respiration and the smaller the proportion which can be utilized in assimilation or which can be accumulated. When tuberization occurs foods are utilized in the construction of the cellular framework of the tubers and accumulate within the cells. If the excess of food remaining after consumption of that portion of the photosynthate utilized in

respiration and in assimilation in the non-tuberous tissues is relatively small the yield of potatoes will be curtailed. Hence profitable crops of potatoes cannot be grown in relatively warm climates, since the proportion of the food manufactured which can be utilized in tuberization is less than in cooler climates. This statement is not controverted by the fact that potatoes are an important crop in Florida, Texas, and other warm climate regions, since this crop is raised in such areas only during the winter and spring months when comparatively cool weather prevails.

The same principle also applies to other plants although the temperature which will result in the maximum P/R ratio varies considerably with the species. Accumulation of foods in any plant is reduced at relatively high temperatures. In most staple crop plants this means a reduction in yield, since the commercially valuable parts of most species are the food accumulating organs such as seeds, fruits, tubers, or roots.

Analysis of the effect of temperature on the P/R ratio is complicated by the fact that plants in their out-of-door habitats are not exposed to a constant temperature, but to a daily cycle of temperature variations, which varies greatly in its pattern, depending upon climatic conditions and even in individual habitats within any climatic center. A daily alternation between relatively cool night temperatures and moderately high daytime temperatures will result in a greater P/R ratio than if both day and night temperatures are relatively high, because under the former condition night rates of respiration are lower.

A relatively low P/R ratio will not only check accumulation but will also retard or may entirely inhibit assimilation. Hence the growth rate of a plant may be greatly slowed down by a low rate of photosynthesis relative to the rate of respiration. In extreme cases death of the plant may result. The effect of variations in the P/R ratio is illustrated by the results of Nightingale (1933) who grew tomato plants under continuous temperatures of 55° F. (13° C.) 70° F. (21° C.), and 95° F. (35° C.). The plants survived, developed, and stored carbohydrates at both the lower temperatures, indicating that in these two groups of plants the P/R ratio was considerably in excess of one. At 35° C., however, the carbohydrate content of the plants decreased rapidly and many of them died in a relatively short time.

Discussion Questions

1. Would the rate of respiration in the leaves of a maple tree be greater on a cloudy day or a clear day if air temperatures were the same?
2. How can respiration occur at night in leaves in which the stomates are closed?

3. How would you proceed to measure the rate of respiration of green leaves? Evaluate the method chosen for sources of error.
4. What gas is usually lost from green plants during the day? At night? Answer the same questions for a non-green plant. Under what conditions is there no gas exchange between a green plant and its environment?
5. Would the complete elimination of all animal life from the face of the earth alter the relative proportions of CO_2 and O_2 in the atmosphere? Explain.
6. How valid is the belief that it is harmful to keep flowers in a sickroom at night?
7. Compare the effects of temperature upon the processes of respiration and photosynthesis and offer possible explanations for any differences.
8. In a certain experiment corn seedlings which had developed for some time in a dark room were measured for the daily variations in rate of respiration. It was found that the rate per hour was practically constant in spite of the fact that marked variations in temperature occurred during the day. Explain.
9. What are some of the ways in which the respiration of living plant tissue might alter the environmental conditions to which it was subject?
10. What daily variations would you expect to find in the rate of respiration of an attached green leaf under "standard day" conditions?
11. Why do starchy seeds decrease proportionately more in dry weight during germination than oily seeds?

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CHAPTER XXX

ANAEROBIC RESPIRATION AND THE MECHANISM OF RESPIRATION

Oxidizing-reducing Enzymes.—A number of substances are known to occur in cells which catalyze oxidations and reductions. Some of these seem to possess all of the attributes of enzymes and are properly so classified. Others are considered to be enzymes by some, but not by all authorities, while some are clearly non-enzymatic in their action. Many of the oxidizing-reducing enzymes operate simultaneously on two different substrates, one of which is oxidized, while the other is reduced. The following discussion of oxidizing-reducing enzymes attempts to represent the generally accepted consensus of most workers in this field at the present time.

Enzymes are conventionally classified into two distinct groups, the hydrolytic enzymes (Chap. XXVII), and the oxidizing-reducing enzymes at present under discussion (Table 55). In a condensed classification it is possible to present only the most probable interpretation of the action of these enzymes in the light of present knowledge. Ideas on this subject are very much in a state of flux, and future investigations may radically change present concepts of the rôle of some of these enzymes.

As a rule the enzymes classed in this group possess the properties as described for enzymes in general in Chap. XXVII. Unlike other enzymes, however, some of the peroxidases are thermostable, which has led some authorities to the view that they are not enzymes in the strict sense of the word.

The occurrence and general properties of these types of enzymes will be discussed briefly.

1. *Zymase* was long considered to be a single enzyme, but is now recognized to be a complex of several enzymes. Zymase is the enzyme system which catalyzes the well known reaction which occurs during alcoholic fermentation, and results in the production of alcohol and carbon dioxide from certain sugars. Most investigations have been made upon zymase extracts prepared from yeast cells, but this enzyme complex is known to be widely distributed through the plant kingdom. Among the enzymes of the zymase complex are *glycolase* which converts hexoses into methyl glyoxal, and *carboxylase* which splits out carbon dioxide from certain types of organic acids (α-ketonic acids),

such as pyruvic acid, forming aldehydes. Equations representing these reactions are given later in this chapter.

TABLE 55—CLASSIFICATION OF THE PRINCIPAL OXIDIZING-REDUCING ENZYMES AND ENZYME SYSTEMS

Enzyme	Substrate	End products
1. Zymase (complex of glycolase, carboxylase, and others)	Glucose, fructose, mannose, galactose, etc.	CO_2 and ethyl alcohol
2. Oxygenases	Organic compound + O_2	Organic peroxide (or organic compounds + H_2O_2)
3. Peroxidases	Phenol compounds + H_2O_2 (or organic peroxide)	Oxidized phenol compounds + H_2O (or reduced organic peroxide)
4. Oxidases (oxygenase + peroxidase) A. Phenolases B. Tyrosinases		
5. Dehydrogenases	Hydrogen donator Hydrogen acceptor	Oxidized donator Reduced acceptor
6. Catalase	H_2O_2 (or organic peroxide)	$H_2O + O_2$ (or reduced peroxide + O_2)

Zymase will not act as a catalytic agent except in the presence of certain co-enzymes, as shown by the following experiment. If an extract of zymase is prepared and then filtered through an ultrafilter it will be found that the enzyme-containing residue on the filter will not, when redispersed in water, catalyze the alcoholic fermentation of a hexose. Neither will the filtrate. If, however, some of the filtrate is mixed with the enzyme the reaction will proceed in the usual way. Evidently there is associated with zymase some diffusable substance or substances which must be present for the operation of this enzyme complex as a catalyst. Further investigation has shown that there are apparently two of these filterable co-enzymes of zymase, one of which is definitely known to be a phosphate, the other apparently being a crystalloidal organic compound.

2. *Peroxidases* are enzymes which bring about the oxidation of phenolic compounds such as pyrogallol, catechol, and benzidine in the presence of hydrogen peroxide. In plant tissues such enzymes may also operate in the presence of organic peroxides. The usual method of demonstrating the presence of a

peroxidase is to bring the plant extract or tissue to be tested into contact with an alcoholic solution of gum guaiacum in the presence of hydrogen peroxide. If a peroxidase is present the solution of gum guaiacum will turn blue in color. This is due to the formation of a blue oxidation product of the guaiacol (a phenolic compound) which is a constituent of the gum guaiacum. Peroxidases are supposed to operate by splitting the peroxide into water and active oxygen, the latter combining with the compound which is oxidized. Peroxidases are of extremely widespread occurrence throughout the plant kingdom, having been found in practically every plant which has been tested for their presence.

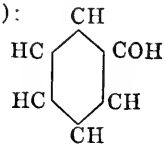
3. *Oxidases.*—It is common observation that when a potato tuber is cut, the exposed tissues usually turn a brownish color. Similar reddish, brownish, or blackish colorations can be discerned in many other plant tissues after cutting or crushing. The frequently observed darkening of plant juices as they are pressed out of a tissue is another example of this phenomenon. Such colorations of plant tissues upon the ready access of atmospheric oxygen are due to the oxidation of certain cell constituents which is brought about by enzyme systems called *oxidases.*

Chodat and Bach (1903) postulated that an "oxidase" actually represents a system composed of two enzymes—an *oxygenase* and a peroxidase. This view has been concurred in by most subsequent workers although there is no general agreement regarding the exact manner in which the two components of this enzyme system operate.

In general the oxygenase is supposed to produce hydrogen peroxide or organic peroxides by the oxidation of certain compounds, using atmospheric oxygen in this process; or, according to another view, to become oxidized itself in the presence of atmospheric oxygen, forming a peroxide. According to Onslow and Robinson (1926) *catechol* and similar compounds are oxidized in the presence of oxygenase, hydrogen peroxide being one of the products of the reaction. Such compounds are known to be of widespread distribution in the plant kingdom.

The peroxidase component of the system is supposed to accomplish the oxidation of phenolic compounds [1] by catalyzing the transfer of oxygen from the peroxide to the compound which is oxidized, as previously described.

[1] Derivatives of phenol (carbolic acid):



Regardless of the precise mechanism by which oxidases operate it is evident that these enzyme systems can accomplish oxidations at the expense of atmospheric oxygen. While peroxidases appear to be of universal occurrence in plants, oxygenases are of more limited distribution. Obviously only those plant tissues which contain both will exhibit oxidase activity.

A plant tissue can be tested for the presence of an oxidase system by dropping a small portion of the macerated tissue into an alcoholic solution of gum guaiacum. Development of a blue color, due to the oxidation of guaiacol, indicates the presence of oxidase. The test is the same as that employed for peroxidases except that hydrogen peroxide is not added.

Two types of oxidases are generally recognized. The *phenolases* seem to be the more widely distributed of the two. Enzymes of this type are found in many of the higher plants, in certain fungi, and in some of the invertebrate animals. Each of the phenolases can catalyze the oxidation of one or more phenol compounds. Among these are such substances as the cresols, toluidins, pyrogallol, phloroglucinol, resorcinol, phenolphthalein, hydroquinone, and guaiacol. The first enzyme of this type to be discovered was found in the juice of the lac tree (*Rhus vernicifera*). This enzyme was called laccase, and the phenolases as a group were formerly called *laccases.*

When the fruiting bodies of some species of fungi are exposed to the air they produce a blue pigment, while those of other species turn at first pink or red and later black. The bluing of fungous tissues is due to the effect of phenolases. The pink and red colorations result, however, from the operation of another oxidase known as *tyrosinase.* This enzyme acts on tyrosine, an amino acid containing a phenol group, and other chemically related substances, oxidizing them through several intermediate stages to the black pigment *melanin.* Intermediate steps in this oxidation process exhibit a series of colors ranging from pink to red to violet, and finally to black. Tyrosine is not oxidized by the phenolases. This enzyme occurs not only in the fungi but in the tissues of many of the higher plants, and in some animals as well.

4. *Dehydrogenases.*—Many biological oxidations and reductions involve intermolecular transfers of hydrogen rather than of oxygen. Enzymes which catalyze such transfers of hydrogen are called *dehydrogenases, oxido-reductases,* or *reductases.* Apparently there are a number of different enzymes of this general type and considered as a group they are widely and probably universally distributed in living organisms.

The action of a typical dehydrogenase can be observed by dropping a block of fresh potato tuber tissue into a dilute solution (about 0.025 per cent) of methylene blue under such conditions that oxygen does not have free access to the tissue or solution. The methylene blue gradually becomes

reduced to a colorless compound called leucomethylene blue. Reduction of the methylene blue is due, not to removal of oxygen from the molecule, but to a transfer of hydrogen from molecules of some other substance, which is termed a *hydrogen donator*, to the molecules of methylene blue, which acts as a *hydrogen acceptor*. Oxidation of the donator molecules thus occurs simultaneously with the reduction of the acceptor molecules (methylene blue). If air is now bubbled through this colorless liquid, the original blue color will return within a short time due to oxidation (*i.e.* dehydrogenation) of the leucomethylene blue back to methylene blue. These two reactions are indicated by the following schematic equations:

$$\underset{\text{Hydrogen donator}}{DH_2} + \underset{\text{Methylene blue}}{Mb} \xrightarrow{\text{Dehydrogenase}} \underset{\text{Oxidized donator}}{D} + \underset{\text{Leucomethylene blue (reduced)}}{MbH_2}$$

$$MbH_2 + \tfrac{1}{2}\,O_2 \rightarrow Mb + H_2O$$

As a specific example of this type of reaction we may take the oxidation of succinic acid to fumaric acid which proceeds according to the following equation:

$$\underset{\text{Succinic acid}}{\begin{matrix}CH_2COOH\\|\\CH_2COOH\end{matrix}} + Mb \xrightarrow{\text{Dehydrogenase}} \underset{\text{Fumaric acid}}{\begin{matrix}CHCOOH\\\|\\CHCOOH\end{matrix}} + MbH_2$$

Methylene blue, which is commonly used in testing for dehydrogenases, is not a natural constituent of living cells; neither are some of the other reagents which have been used in testing for such enzymes. However living organisms apparently contain a number of naturally occurring compounds which act in a manner analogous to methylene blue, *i.e.* as hydrogen acceptors. Among these are the so-called "respiratory pigments" which are known to occur in many plant tissues and the compounds *cytochrome* and *glutathione* which have been found to be of widespread occurrence in both plant and animal tissues (see later).

Similarly, it has been found that living organisms contain a number of compounds which can serve as hydrogen donators in reactions similar to that just described. Among these are xanthine, fatty acids, aldehydes, sugars, and amino acids.

5. *Catalase* is one of the most ubiquitous of all enzymes and has been found in practically all plant and animal tissues which have been tested for its presence. This enzyme activates the reduction of hydrogen peroxide into water and molecular oxygen according to the equation:

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Since the oxygen released is in the molecular rather than the active state, it cannot be utilized in oxidations. It is also supposed that catalase can liberate molecular oxygen from organic peroxides. The presence of catalase in plant or animal tissues can be demonstrated by bringing some of the tissue into contact with a dilute solution of hydrogen peroxide. If a small block of a potato tuber, for example, is dropped into a test tube containing hydrogen peroxide solution, evolution of bubbles of oxygen ensues immediately.

This enzyme can be determined quantitatively by carrying out the reaction in an apparatus in which the rate of evolution of oxygen can be determined. Because of the ease with which such quantitative measurements of catalase activity can be made, many extensive studies of this enzyme have been carried out.

Considerably more time and attention have been devoted to precise studies of the occurrence and action of catalase than the very inadequate knowledge of its rôle in living organisms would seem to warrant. Despite its almost universal presence in living cells, the metabolic significance of this enzyme, if any, is obscure. It has been suggested that catalase may act as a sort of "safety valve" by preventing the accumulation in cells of hydrogen peroxide or other peroxides produced as a result of cell metabolism. There is, however, no positive evidence in support of this suggestion.

Metabolically active plant tissues usually exhibit a high rate of respiration and a high general level of enzymatic activity. Hence a correlation usually exists between the catalase activity of a tissue and its metabolic status. Measurements of the catalase activity of a tissue are therefore often accepted as an index of the intensity of metabolic activity in that tissue.

Fermentation.—Of all the many known types of fermentations by far the most important, both from the theoretical and practical point of view, is the process of alcoholic fermentation. Although this process has been familiar to the human species for time out of mind, it was not until the classical researches of Pasteur, begun in 1857, that it was recognized that alcoholic fermentation resulted from the metabolic activities of yeast plants. Yeasts, it should be recalled, are single-celled organisms belonging to the *Ascomycetes*. Yeasts may multiply by budding and under certain conditions produce *ascospores* (Fig. 116).

Pasteur also realized that alcoholic fermentation was an anaerobic process and in fact characterized fermentation as "life without oxygen." The final basic step in the understanding of the alcoholic fermentation was furnished by Buchner's demonstration in 1897 that an active agent or enzyme—zymase—could be extracted from yeast cells, and that this enzyme could catalyze the process in the total absence of yeast cells.

Alcoholic fermentation will occur in almost any moist sugar-containing medium or sugar solution which is inoculated with yeast or which is left exposed to the air. Since various species of wild yeasts are blown about through the atmosphere inoculation of such media will occur without human intervention.

The following summary equation represents the chemical changes occurring in alcoholic fermentation:

$$C_6H_{12}O_6 \xrightarrow{\text{Zymase}} 2\ C_2H_5OH + 2\ CO_2 + 25\ \text{kg.-cal.}$$

As this equation shows, fermentation of one mol of a hexose sugar results in the production of two mols of ethyl alcohol and two mols of carbon dioxide, energy to the amount of approximately 25 kg.-cal. being released in the process. The carbon dioxide evolved escapes as a gas accounting for the effervescence of a fermenting liquid. Certain by-products such as glycerol, succinic acid, amyl alcohol and other compounds are also usually produced as a result of subsidiary reactions.

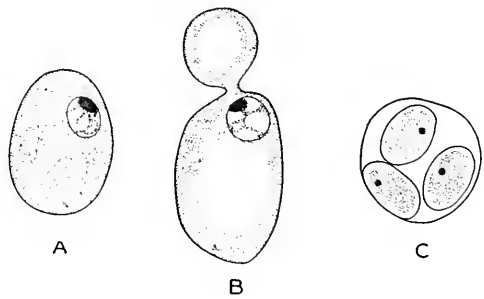


FIG. 116. Yeast plants (*Saccharomyces cerevisiae*). (*A*) a vegetative cell, (*B*) early stage in vegetative multiplication of a yeast cell, (*C*) ascus containing ascospores. Redrawn from Gäumann and Dodge (1928) after Guillermond.

Undoubtedly the reaction as given above occurs in a number of steps, and certain intermediate products are formed by the disintegration of the sugar molecules which are subsequently converted into the compounds which finally appear as the end products. Since zymase is known to be a complex of several enzymes it seems probable that different stages in the process of alcoholic fermentation proceed under the influence of different components of this enzyme system.

Various other types of fungi besides the yeasts can accomplish alcoholic fermentation. As we shall see shortly a process also occurs in green plants which is at least closely analogous to, and may be identical with alcoholic fermentation.

Yeasts can ferment glucose, fructose, galactose and mannose directly. Since yeast cells also produce the enzymes sucrase and maltase, the disaccharides sucrose and maltose can also be fermented after being hydrolyzed to hexose sugars. On the other hand yeast cells cannot ferment starch because

they produce no amylase. This is the reason that germinated barley (malt) is used rather than the ungerminated grains in the brewing industry, since the sugar content of the grains increases greatly during germination.

Alcoholic fermentation is an anaerobic process, occurring without any utilization of atmospheric oxygen. Oxidation is accomplished by intermolecular atomic shifts which take place in such a manner that the sum total of the energy remaining in the resulting compounds is less than that present in the original substrate, the excess energy being released. Alcoholic fermentation results in only an incomplete oxidation of hexose molecules, hence the quantity of energy released—about 25 kg.-cal. per mol of glucose—is much less than in aerobic respiration, in which oxidation of one mol of glucose sets free 673 kg.-cal. In spite of its relative inefficiency the process of fermentation is the method by which yeast plants obtain necessary energy. This is the fundamental biological significance of the process.

It might be supposed that efficient aeration of a sugar solution containing yeast plants would result in complete oxidation of the sugars present by a process of aerobic respiration. Such, however, is not the case; ethyl alcohol and carbon dioxide are the principal end products whether the reaction occurs in the presence or absence of oxygen. Some aerobic respiration, (as much as one-third of the total, according to some investigators) does occur when oxygen has access to the yeast cells. The predominance of anaerobic respiration even in the presence of oxygen is generally ascribed to the possession by yeast cells of a relatively ineffective oxidizing enzyme mechanism, as compared to a highly active zymase system. In the presence of oxygen multiplication of yeast cells occurs at a more rapid rate than in its absence. This is probably due to the much greater energy production resulting from the occurrence of some aerobic respiration when oxygen is available.

When sugar in solution is fermented by yeast one of the end products —ethyl alcohol—accumulates in the solution, while the other—carbon dioxide —escapes as a gas. However, there is a definite limit to the accumulation of alcohol. When the proportion of alcohol in the liquid reaches about 15 per cent the yeast cells are poisoned and the fermentation process stops.

Anaerobic Respiration.—Any higher animal which is deprived of oxygen will die within a very few minutes. Higher plants, however, will continue to live in an atmosphere devoid of oxygen. Some plants or plant organs can survive under such conditions for a long period, others succumb within a day or two. Corn (maize) seedlings, for example, will not remain alive much more than a day in an oxygen-free atmosphere. On the other hand, apple and pear fruits can survive storage in an atmosphere of pure hydrogen or pure nitrogen for months without injury. Even under such conditions

plants continue to evolve carbon dioxide, thus indicating the occurrence of respiration. Respiration of this type, proceeding in the absence of atmospheric oxygen, is called *anaerobic respiration*. The principal conditions under which anaerobic respiration may or does occur in tissues of higher plants will be discussed briefly.

Many species of plants are adversely affected by flooding of the soils in which they are rooted (Chap. XVII). Submergence of bottomland corn fields—a frequent occurrence in some regions—soon results in serious damage to the plants. This takes place even if only the roots are immersed. Maintenance of a saturated condition in the soil in which corn (maize) plants are rooted for only a few days results in a yellowing and marked stunting of the plants, and persistence of a flooded condition of the soil for a much longer period soon results in their death. The plants often exhibit many of the symptoms of desiccation, showing that the normal physiological processes of the roots have been disturbed, and that absorption of water is no longer occurring at an adequate rate.

The roots of maize apparently require a relatively high partial pressure of oxygen in the soil atmosphere for the maintenance of aerobic respiration. When the supply of atmospheric oxygen to the roots is cut off by inundation of the soil, anaerobic respiration replaces aerobic respiration. Possible causes of the detrimental effect of continued anaerobic respiration on plant cells will be considered later.

Most of the other known examples of anaerobic respiration in plants result from structural features of plant organs which prevent ready access of oxygen to interior tissues. For example, a number of species produce seeds with coats which are only slightly permeable to oxygen. During the earlier stages of the germination of such seeds, before the coats are ruptured, respiration is largely of the anaerobic type. The best known example of this is in pea seeds, which in the early stages of germination evolve a volume of carbon dioxide three or four times as great as the volume of oxygen absorbed. Similarly the respiration occurring in corn grains, oat grains (especially if the glumes are left intact) and sunflower fruits during the early stages of germination is largely anaerobic (Frietinger, 1927). A similar condition probably obtains in many other seeds and dry fruits.

Anaerobic respiration occurs naturally in some fleshy fruits. The "skin" of some fruits, of which the grape is the most familiar example, is relatively impermeable to oxygen, hence some anaerobic respiration undoubtedly occurs in such organs. Somewhat similar conditions prevail in the ripening fruits of the Japanese persimmon.

It has been rather generally supposed that the interior tissues of most

bulky fruits such as bananas, citrus fruits, melons, etc. often suffer from a deficiency of oxygen and that anaerobic respiration is of frequent occurrence in such tissues. (Gustafson, 1930) has shown that tomato fruits respire anaerobically when enclosed in an atmosphere of nitrogen or hydrogen and considers it a possibility that they may carry on some anaerobic respiration even when exposed to a normal atmosphere.

On the other hand analysis of the internal atmosphere of some of the cucurbitaceous fruits has shown the oxygen concentration to be almost as high as in the atmosphere. While it is impossible to draw a definite conclusion regarding the prevalence of anaerobic respiration in fleshy fruits, it seems probable that this process occurs in at least some fruits of this type.

Cacti will respire anaerobically when enclosed in an atmosphere of pure nitrogen (Gustafson, 1932) and it seems probable that the deep-seated tissues of succulent species may respire anaerobically under natural conditions.

External factors appear to influence the rate of anaerobic respiration in much the same way that they affect respiration of the aerobic type. The influence of temperature follows a time factor pattern similar to that found for aerobic respiration. For germinating pea seeds the optimum temperature (temperature at which the rate is maintained for a long time) is about 30° C. (Fernandes, 1923). Various toxic substances appear to influence both types of respiration in a similar manner.

The Similarity between Anaerobic Respiration and Alcoholic Fermentation.—Considerable evidence exists that the process of anaerobic respiration as it occurs in the higher green plants is identical with, or at least very similar to the process of alcoholic fermentation as carried on by yeasts and some other micro-organisms. The same summary equation is therefore supposed to represent both processes. There are three main reasons for believing that these two processes are essentially similar:

1. Hexose sugars are usually the substrate which is oxidized in both alcoholic fermentation and anaerobic respiration.

2. The principal end products—carbon dioxide and ethyl alcohol—are also the same in the two processes. We have already seen that plant tissues evolve carbon dioxide when subjected to anaerobic conditions. Alcohol also accumulates in the tissues of higher plants during anaerobic respiration. The quantities of alcohol produced in most plant tissues during anaerobiosis are less than would be theoretically expected in terms of the generally accepted equation. A probable explanation of this fact is that other compounds besides alcohol are produced as end products in this process as it occurs in the higher plants. This does not necessarily invalidate the fundamental concept of an essential similarity between anaerobic respiration and alcoholic fermentation.

Alcohol may be produced in the process in a quantity equivalent to that indicated by the equation, but part of it may be immediately converted into other compounds by subsidiary reactions. Another possibility is that in the higher plants the process takes a slightly different course, certain other products being formed in addition to or instead of ethyl alcohol.

3. The complex of enzymes known as zymase is present in the cells of a number of species of the higher plants as well as in yeast (Stoklasa and Czerny, 1903). It is now considered that this enzyme is of widespread and probably of universal occurrence in plant cells.

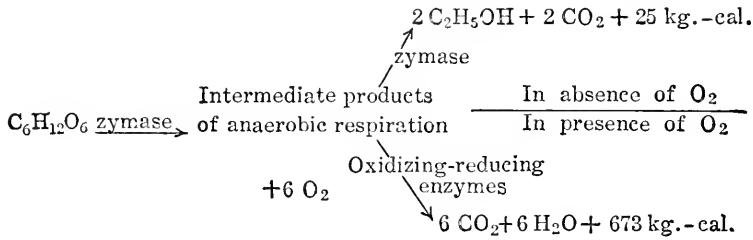
Causes of Injury to Cells as a Result of Anaerobic Respiration.—As has already been noted, prolonged anaerobic respiration has a detrimental or even lethal effect on many plant tissues. At least two probable reasons for this effect have been recognized. As the equations already presented show, the oxidation of one mol of glucose in aerobic respiration results in the liberation of 673 kg.-cal., while the energy released from the same quantity of glucose in anaerobic respiration is only about 25 kg.-cal. Aerobic respiration is therefore more than twenty-five times as effective an energy-releasing process as anaerobic respiration. This is probably one of the reasons why many plant tissues, especially if metabolically active, cannot endure prolonged anaerobiosis. When anaerobic is substituted for aerobic respiration the rate of energy release is inadequate for the maintenance of cell processes, and deleterious effects are soon produced.

A second probable cause of injury during anaerobic respiration is the accumulation of substances toxic to the protoplasm. In anaerobically respiring tissues alcohol and other substances described later usually accumulate in the cells. These compounds are toxic to protoplasm and probably account, at least in part, for the injurious effects produced in many plant tissues by continued anaerobic respiration. It is perhaps significant that senescent tissues such as ripe fruits, in which metabolic activity is sluggish, can usually endure a more prolonged period of anaerobic respiration without injury than metabolically active tissues.

The Relation between Anaerobic and Aerobic Respiration.—There are a number of reasons for believing that a close relationship exists between these two types of respiration as they occur in the higher green plants. In 1878 Pfeffer proposed a theory which involved this concept. He postulated that aerobic respiration takes place in two steps. In the first sugar was supposed to be split anaerobically into alcohol and carbon dioxide and in the second alcohol was supposed to be aerobically oxidized into carbon dioxide and water.

For various reasons this theory proved to be untenable but a modifica-

tion of it, which has been strongly supported by Kostychev (1927), is now rather generally regarded as at least an excellent working hypothesis of the inter-relationship of the two types of respiration occurring in the cells of higher plants. This theory is illustrated schematically in the following diagram:



As this diagram indicates certain enzymes of the zymase complex are first supposed to convert the sugar into labile intermediate products, this anaerobic stage being the first step in both aerobic and anaerobic respiration. After this step the course of the reaction is supposed to depend upon whether or not oxygen is available. If not, the reaction proceeds anaerobically, alcohol and carbon dioxide being produced from the intermediate products, probably as a result of the action of other enzymes of the zymase complex. If oxygen is available, then complete oxidation of the intermediate products to carbon dioxide and water results under the influence of oxidizing-reducing enzymes.

Among the reasons for believing in the essential validity of this theory are:

1. The fact that anaerobic respiration is apparently of universal occurrence in higher plants when deprived of oxygen.

2. The apparently invariable presence of the enzyme zymase in the cells of higher plants.

3. Some of the same intermediate products, as for example acetaldehyde, which are found in the anaerobic respiration of hexose sugars, have also been detected in plants during aerobic respiration. Gustafson (1934), for example, has shown that acetaldehyde apparently is always present in respiring tomato fruits.

4. When seedlings are "fed" fermented sugar solutions a great increase in the rate of respiration can be observed, indicating that certain intermediate products of alcoholic fermentation (hence by inference of anaerobic respiration) can serve as the respiratory substrate.

5. When oxygen is again allowed access to plant tissues which have been deprived of it for some time, a temporary increase in the rate of aerobic respiration can usually be observed. This appears to indicate the accumulation during anaerobic respiration of oxidizable substances in the cells, and that this

increase in the concentration of the respiratory substrate results in a transient acceleration of the rate of respiration.

6. None of the oxidizing-reducing enzymes of plants are able to catalyze the oxidation of sugar directly, but apparently certain of them can bring about the oxidation of the intermediate products of anaerobic respiration to carbon dioxide and water.

The Theoretical Mechanism of Anaerobic Respiration.—The respiratory substrate in the higher green plants is usually a hexose sugar. The two common hexose sugars in plants are glucose and fructose. As discussed in Chap. XXII there is good evidence that both of these sugars can exist in highly reactive forms known as gamma-sugars. Sugars of this type are so highly labile that they have never been isolated in the pure state.

Recent extensive investigations of Neuberg and his co-workers (1922, 1924, etc.) have thrown considerable light on the chemical nature of the intermediate products in alcoholic fermentation. The same or similar products are believed to be formed in the anaerobic respiration of higher plants. The supposed stages of the process are as follows:

1. "Activation" of the stable hexose molecules, or, in other words, conversion of molecules of this type into the labile gamma-sugars. Formerly it was believed that gamma-glucose was the sugar usually oxidized in the respiration process, but recent investigations indicate that gamma-fructose may often be cast in this rôle.

2. The "activated" hexose molecule (*i.e.* gamma-hexose) is split by the enzyme *glycolase* (a component of the zymase complex) into two molecules of methyl glyoxal, with the elimination of two molecules of water as follows:

$$C_6H_{12}O_6 \xrightarrow{\text{Glycolase}} \underset{\text{Methyl glyoxal}}{2\ CH_3\cdot CO\cdot CHO} + 2\ H_2O$$

3. The next step is the conversion of two molecules of methyl glyoxal into one molecule of glycerol and one of pyruvic acid, by a reaction with two molecules of water. The formation of glycerol from methyl glyoxal is a reduction process, the formation of pyruvic acid an oxidation process. A reaction of this type is known as a *Cannizzaro reaction:*

$$\begin{array}{ccc} \underset{\text{Methyl glyoxal}}{CH_3\cdot CO\cdot CHO} & & \underset{\text{Glycerol}}{CH_2OH\cdot CHOH\cdot CH_2OH} \\ + & H_2 + H_2O & + \\ CH_3\cdot CO\cdot CHO & \| \quad \rightarrow & CH_3\cdot CO\cdot COOH \\ \text{Methyl glyoxal} & O & \text{Pyruvic acid} \end{array}$$

This reaction may be catalyzed by an enzyme of the dehydrogenase type, but there is no convincing evidence on this point.

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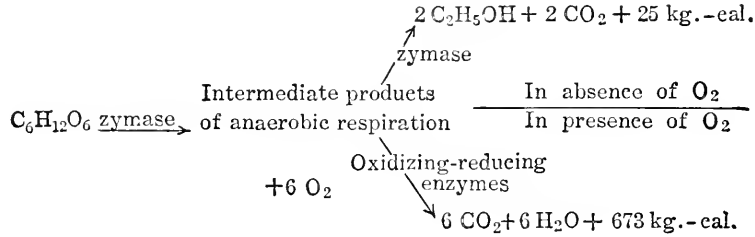

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$$\begin{array}{ccccc} \underset{\text{Methyl glyoxal}}{CH_3\cdot CO\cdot CHO} & & & & \underset{\text{Glycerol}}{CH_2OH\cdot CHOH\cdot CH_2OH} \\ + & & H_2 + H_2O & & + \\ \underset{\text{Methyl glyoxal}}{CH_3\cdot CO\cdot CHO} & & \| & \rightarrow & \underset{\text{Pyruvic acid}}{CH_3\cdot CO\cdot COOH} \\ & & O & & \end{array}$$

This reaction may be catalyzed by an enzyme of the dehydrogenase type, but there is no convincing evidence on this point.

4. The pyruvic acid is then split immediately into acetaldehyde and carbon dioxide, under the influence of *carboxylase*, another enzyme of the zymase complex:

$$\underset{\text{Pyruvic acid}}{CH_3 \cdot CO \cdot COOH} \xrightarrow{\text{Carboxylase}} \underset{\text{Acetaldehyde}}{CH_3 \cdot CHO} + CO_2$$

5. Up to this point the process of respiration is supposed to proceed along the same course, whether it occurs under aerobic or anaerobic conditions. Under *anaerobic* conditions the next step is supposed to be a second Cannizzaro reaction in which a molecule of the methyl glyoxal produced in stage 2 reacts with a molecule of the acetaldehyde produced in stage 4 as follows:

$$\begin{array}{ccccc} \underset{\text{Methyl glyoxal}}{CH_3 \cdot CO \cdot CHO} & & & & \underset{\text{Pyruvic acid}}{CH_3 \cdot CO \cdot COOH} \\ + & + & \overset{O}{\underset{H_2}{\|}} & \rightarrow & + \\ \underset{\text{Acetaldehyde}}{CH_3 \cdot CHO} & & & & \underset{\text{Ethyl alcohol}}{CH_3 \cdot CH_2 \cdot OH} \end{array}$$

The resulting pyruvic acid is supposed to be acted on by carboxylase as in stage 4, but the alcohol is not subject to further chemical change, and becomes, together with the carbon dioxide evolved in stage 4, one of the final end products of the reaction.

In another recently proposed theory of alcoholic fermentation phosphate esters of the carbohydrates are supposed to be intermediate compounds in the reaction rather than methyl glyoxal (Meyerhof and Kiessling, 1935). It is supposed that hexose diphosphates are first formed, that these are converted into a compound composed of a triose combined with phosphoric acid which passes through several intermediate stages, finally resulting in the production of pyruvic acid. Acetaldehyde is then supposed to be formed from pyruvic acid by loss of carbon dioxide, and ethyl alcohol to be formed from the acetaldehyde.

Theories of the Mechanism of Aerobic Respiration.—Up to and including the stage in which "intermediate products" such as acetaldehyde are formed it is generally supposed that the progression of stages in both aerobic and anaerobic respiration is identical. The problem yet remaining before us is that of the steps by which the intermediate products of anaerobic respiration are converted into carbon dioxide and water in the process of aerobic respiration.

Many extensive investigations of the mechanism of biological oxidations have been undertaken upon both plant and animal tissues, and a number of rather diverse opinions have been entertained regarding the nature of cell

oxidations and reductions. In the following brief discussion it will be possible for us to touch on only a few of the more important suggestions which have been advanced regarding the mechanism of the aerobic phase of plant respiration.

Palladin (1909) proposed a theory of aerobic respiration which continues to merit consideration. This theory will purposely be discussed in a somewhat more generalized form than that first proposed by Palladin, and can be represented by the following equations:

$$\underset{\text{Intermediate products of anaerobic respiration}}{C_6H_{12}O_6} + 6\,H_2O + \underset{\text{Hydrogen acceptor}}{12\,A} \xrightarrow{\text{Dehydrogenase}} 6\,CO_2 + \underset{\text{Reduced acceptor}}{12\,AH_2}$$

$$\underset{\text{Reduced acceptor}}{12\,AH_2} + 6\,O_2 \xrightarrow{\text{Oxidase}} \underset{\text{Hydrogen acceptor}}{12\,A} + 12\,H_2O$$

In the first stage of the process the intermediate products of anaerobic respiration (acetaldehyde, etc.) are supposed to be oxidized by the active oxygen remaining after the dehydrogenation of water, the hydrogen released combining with a hydrogen acceptor (A). Since the exact chemical nature of the intermediate products of anaerobic respiration is uncertain, representation of this reaction is simplified by indicating them by the formula $C_6H_{12}O_6$. Such a reaction is probably catalyzed by a dehydrogenase. The oxygen utilized does not come from the atmosphere, but from the water molecules.

In the second stage of the process the reduced hydrogen acceptor (AH_2) is supposed to be oxidized to its original condition. This reaction is presumably catalyzed by oxidases which as a system possess the capacity of activating atmospheric oxygen. According to this view atmospheric oxygen is consumed, not in direct oxidation of the substrate, but in the oxidative regeneration of the hydrogen acceptors.

In Palladin's own formulation of this theory the rôle of hydrogen acceptors was ascribed to certain types of pigments found in plants. These he termed *respiratory pigments*. In the first stage of the process the respiratory pigment is supposed to be reduced to a colorless form or *chromogen* (the behavior of methylene blue, previously described, is analogous), while in the second stage this reduced pigment or chromogen is oxidized back to the pigment. Palladin's theory, in the restricted form in which he proposed it, has often been called the *chromogen theory* of respiration.

Pigments which can be shown experimentally to react in the manner postulated by Palladin have actually been isolated from the tissues of some plants,

and doubtless occur in many others. Such pigments invariably seem to be phenol compounds which is in accord with the fact that oxidases apparently can oxidize only substances containing a phenol grouping.

However, it is not certain that respiratory chromogens occur in all plant tissues, and it is well known that all plant tissues do not contain oxidases. That this theory, in its original restricted form, is universally applicable to plants is therefore doubtful. The possibility is by no means precluded, however, that other substances besides respiratory pigments may serve as hydrogen acceptors in the respiratory process. Indeed, the results of recent investigations indicate that this is very probable. The widespread occurrence of *cytochrome* and *glutathione* in plant and animal tissues has already been mentioned. Both of these substances can serve as hydrogen acceptors. After combining with hydrogen they can be oxidized back to their original state by the loss of hydrogen; *i.e.* can also serve as hydrogen donators. Such compounds might therefore act as hydrogen acceptors in a respiratory mechanism otherwise essentially similar to that proposed by Palladin. Other compounds possessing similar properties may also occur in living cells. Some such substances may be able to accomplish oxidations and reductions without the intermediation of enzymes, but the evidence upon this point is not yet clear.

While theories of the mechanics of aerobic respiration of this general type have by no means been fully substantiated, they do serve as provocative hypotheses and are in general accord with present factual knowledge.

Comprehensive investigations have been carried out in recent years by Blackman and his associates on aerobic and anaerobic respiration in apple fruits. As a result of these studies certain hypotheses regarding the mechanism of respiration have been proposed (1928). In general the conclusions drawn from the results of these investigations agree with the theories of respiration which have just been described. Two additional points are stressed by Blackman, however. In the first place oxygen appears to be required, not only in the oxidation of the intermediate products of anaerobic respiration, but also seems to have an important effect in the "activation" of the hexose molecules. In other words, with increase in the availability of oxygen the rate of conversion of hexoses into an active form (presumably gamma-glucose or gamma-fructose) is increased. The second additional point brought out by Blackman's investigations is that not all of the carbon in the compounds formed as a result of the action of the zymase complex (*i.e.* the intermediate products of anaerobic respiration) is oxidized to carbon dioxide. Since, according to Blackman, about three-fourths of the carbon in these compounds is not oxidized it is obvious that a large proportion of the products of zymase activity must remain in the cells. According to Blackman these products are

worked back into the cell system, a process which he terms *oxidative anabolism*. The course which such transformations in the products of zymase activity follow is unknown. Genevois (1927) has come to similar conclusions as a result of studies on the process of respiration in sweet peas.

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CHAPTER XXXI

GROWTH, ASSIMILATION, AND ACCUMULATION

That plants more or less continuously increase in size and produce new organs at least intermittently throughout their life history is one of the most self-evident of natural phenomena. The term "growth" is popularly employed to designate this complex of processes, and in a loose sense, at least, is so employed by botanists. Growth is the one plant process with which few persons are unfamiliar, even if they have never observed it on any larger scale than a potted plant on a window sill. For farmers, horticulturists, foresters, and all others who depend upon the productivity of plants for their livelihood the phenomenon of plant growth holds the center of the stage of interest.

In many discussions of plants as living organisms emphasis is laid upon the "structure" and "function" of various organs and tissues. Most such discussions overlook or at least fail to emphasize that structure is also a result of "function" if this latter term is considered to refer to physiological activity. The coordinated development or growth of plant organs and tissues is just as clearly a form of physiological activity as such relatively simpler processes as photosynthesis and respiration. However, because of the complexity of the process, the physiology of growth has been studied much less intensively than the end products—cells and tissues—of growth activity.

Assimilation.—The dry matter which is incorporated into the structure of both protoplasm and cell walls during growth comes almost entirely from foods. The process whereby foods are utilized in the building of protoplasm is often called *assimilation*. For convenience in discussion we will use this term in a blanket sense to refer to the construction of both protoplasm and cell walls from foods. In the synthesis of protoplasm the foods assimilated are largely proteinaceous, while those assimilated in the fabrication of cell walls are almost entirely carbohydrates. The chemical reactions involved in assimilation are principally condensations in which simple, soluble foods are converted into complex, insoluble constituents of cell systems. These reactions are probably catalyzed by enzymes. As a result of assimilation a growing region invariably increases in dry weight during growth.

Apparent exceptions to the principle of increase in dry weight during growth are sometimes cited. A seedling developing in the dark, as described

in Chap. XXIX, although obviously growing, continuously decreases in total dry weight. Even when seeds germinate in the light the total dry weight of the plant decreases for a period prior to the initiation of photosynthesis in the developing seedling. Similarly, for a short time in the spring, when the buds of woody plants resume growth, a slight decrease occurs in the total dry weight of the plant. Likewise an actively growing plant steadily decreases in total dry weight during the night hours. Such examples, however, only obscure the crucial fact that the *growing region* invariably increases in dry weight during the process of growth. In such examples as those cited assimilation of foods in the growing regions is proceeding at the expense of accumulated foods, and the high respiratory rate which is also an accompaniment of growth results in the oxidation of foods and the resultant loss of total dry weight. Even under such conditions the plant is increasing in terms of *assimilated* dry weight.

Meristems.—In general growth is initiated in tissues in which cell divisions can occur. The primordial tissue of every growing region is a meristem which, loosely defined, is a tissue in which some or all of the cells possess the capacity of cell division. While it is evident that a meristem must be an integral part of every growing region of a plant, its rôle in the growth process is a restricted one, since it only sets in operation the first readily observable step in the complex series of processes which are usually included under the term of growth.

Meristems sometimes arise *de novo* from living cells of the plant but most such regions present at any time in the body of a plant represent the current generation of an unbroken lineage of meristematic cells extending back to the cell—usually a fertilized egg cell—from which that plant body originated. The important meristems of which this is usually true are the apical meristems at stem and root tips and the cambium. Differentiation of the initial root meristem, the initial stem meristem, and the cambium occurs very early in the ontogeny of any individual plant. Determination of the general organization of the plant body—which is a basic and integral phase of the growth process—thus occurs very early in its life history.

Growth which is initiated in the apical stem and root meristems is called *primary growth*. Primary growth results in the construction of the *primary tissues* of a plant, accounts for all increase in length of the plant axis at both stem and root tips, results in the development of the branching system of the stem and roots, and is responsible for the production of lateral appendages such as root hairs, leaves and floral parts.

In many species the primary tissues constitute the entire plant. This is true of the ferns and their allies, and of most monocots. In gymnosperms and most dicots, however, stems and roots not only grow more or less con-

tinuously by proliferation of the fundamental tissues of which these organs are composed, but also increase in diameter as a result of cambial activity. The tissues developed as a result of cambial activity, together with those developed by certain special types of meristems such as cork cambiums, are called *secondary tissues*.

Some increase in diameter may occur, however, even in stems and roots which do not possess a cambium. Increase in the girth of a young stem as a result of primary growth may continue for some time after increase in length of that region of the stem has ceased, due to a slow continuance of cell division and enlargement in some of the tissues, particularly those near the periphery. In species in which the primary tissues constitute the entire body of the plant this is the sole mode of growth in diameter.

Dynamics of the Growth Process.—Growth almost invariably involves not only a progressive increase in the dry weight of the growing region, but a series of differentiation phenomena which become increasingly complex during the growth process. Physiological differentiation of the protoplasm begins before cell division or any other observable manifestations of growth can be detected. Such differentiation undoubtedly continues throughout most or all of the growth process but is sooner or later accompanied by other kinds of differentiation such as size and shape differentiation of cells, structural and chemical differentiation of cell walls, etc. The organized tissue systems of mature plant organs are developed as a result of the coordinated differentiation of cells during the growth process.

The dynamics of growth will be described principally as exemplified by the apical stem meristem of a dicot. Under a microscope examination of a section cut longitudinally through a stem tip reveals three easily distinguishable but intergrading regions usually termed (1) the *region of cell division* which includes the so-called *promeristem,* (2) the *region of cell enlargement,* and (3) the *region of cell maturation* (Fig. 117). These regions are usually considered to correspond to the three principal morphological phases of growth.

The developmental stages through which the cells at any level of the stem axis pass can thus be found at any given moment in successive levels of the axis, each in turn farther away from the apex. The appearance of the cells at progressively lower levels back of the stem terminus indicates the procession of modifications which the apical cells of that stem tip would normally have undergone during its elongation, had their usual destiny not been interrupted by preparing the tissue for miscroscopic examination.

Such sections represent only the appearance of the cells through one longitudinal plane of a growing stem tip at one particular instant in its history. They can no more afford any adequate representation of the kalei-

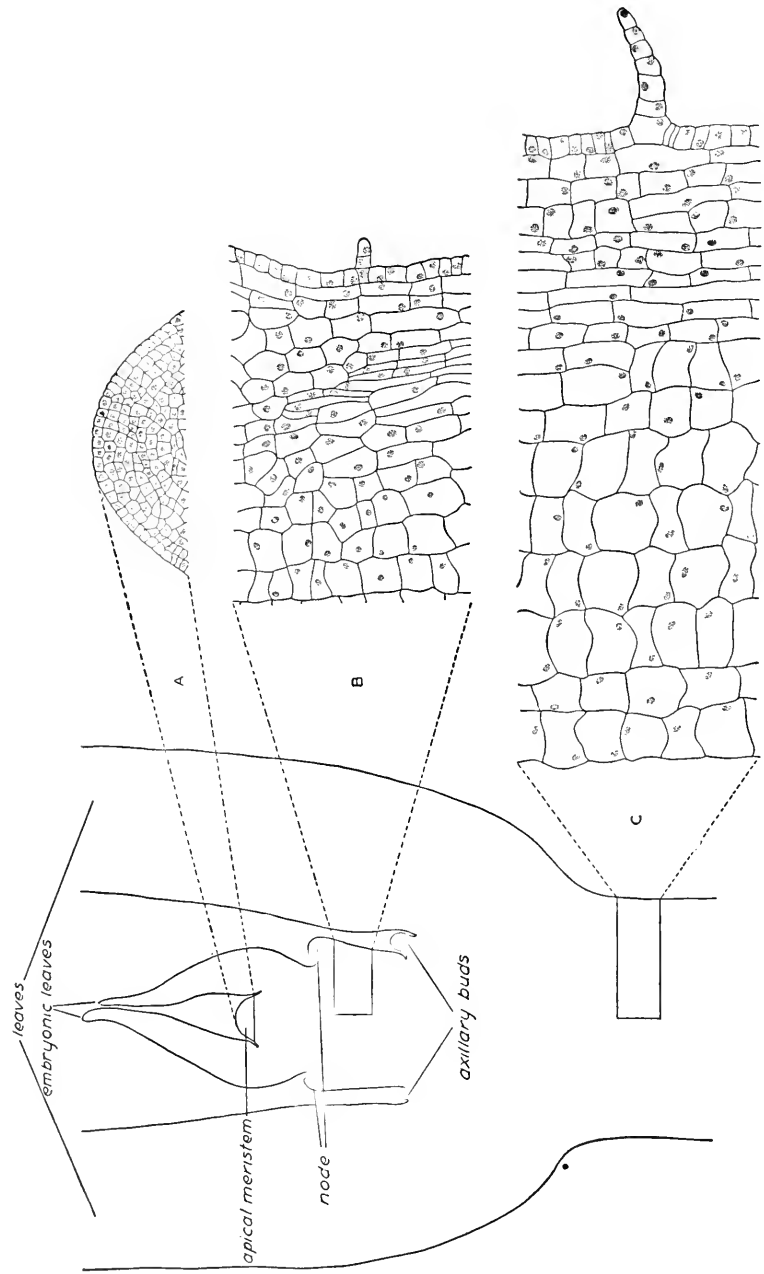

FIG. 117. Outline longitudinal section through stem tip of *Coleus blumei*. (*A*) detail of apical meristem, (*B*) detail of zone in which cell enlargement is predominant phase of growth, (*C*) detail of zone in which maturation of some tissues is largely completed.

doscopic growth process than a snapshot can convey any adequate impression of the colorfulness and strategy of a championship football game. Such sections picture us only the results of growth activity, but nothing of the dynamics of the process.

1. *The Cell Division Phase.*—Every vegetative stem tip contains one or more cells called *initials,* the position of which is maintained at or near the apex. All new cells produced at any growing stem or root tip originate directly or indirectly, after intervening cell divisions, from these persistently meristematic cells. In pteridophytes there is generally only one initial, but in angiosperms a number of initial cells are usually present in the growing tip (Fig. 118).

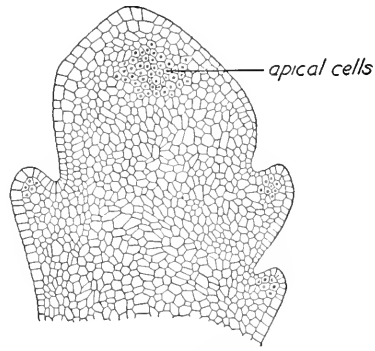

FIG. 118. Longitudinal section through stem tip of grape (*Vitis*) to show apical cells (initials). Redrawn from Eames and MacDaniels (1925).

One of the two daughter cells originating upon the division of an initial retains its position and meristematic properties. From several to many generations of cell progeny may arise from the other, but eventually all of the offspring of this daughter cell become mature cells except any which are so located that they become cambium cells. Cell division in apical meristems occurs both longitudinally and in many planes at right angles to the axis, thus giving rise to the typical cylindrical configuration of a stem or root tip. Multiplication of cells during growth results not only in an increase in the number of cells present but also in some increase in the size of the growing organ, since the combined volume of two daughter cells is usually greater than that of the mother cell.

The youngest cells of a meristem are called the *promeristem.* The cells of the promeristem, which includes the initials, are distinguished by certain structural features. Such cells, if observed in the non-dividing condition, are seen to possess thin, delicate walls and are usually approximately isodiametric. The mass of cells in any meristem appears to be in a highly plastic condition and its continuity is not interrupted even by minute intercellular spaces. There is also some evidence that the intervening walls are actually impregnated with protoplasm. Due to the relatively small size of the cell the nucleus is relatively prominent, although actually it is no larger than in mature cells. The cells of the promeristem are often described as being non-

vacuolate, but all such cells which have been adequately studied have been shown to contain minute vacuoles (Zirkle, 1937).

The cells in the older portions of the meristem differ in certain respects from those of the promeristem. Priestley (1929) distinguishes these as "vacuolating and dividing cells." Cell divisions continue in this zone of the meristem at the same time that the vacuoles are increasing appreciably in volume. Some increase in the size of the cells occurs during this period in their growth and the walls become distinctly thicker. Intercellular spaces also appear at this stage and are at first filled with a dilute sap.

The zone of cell division in any actively growing region is a center of intense assimilatory activity. Since every cell formed as a result of mitosis contains its own complement of protoplasm and since every cell division involves the formation of a cross wall between the two daughter cells as well as some extension of existing walls, both carbohydrates and proteinaceous foods are assimilated during cell division. Cellulose, pectic compounds, and other cell wall constituents are produced by the condensation of the molecules of simple soluble carbohydrates. Protoplasmic proteins are formed principally by the condensation of amino acids which are probably transported to the meristem as such or which may, at least in some species or under some conditions, be synthesized in the meristematic cells from carbohydrates and nitrogenous compounds.

In the promeristem, at least, the quantity of protoplasm built up is proportionately very large as compared with the quantity of new cell wall material constructed. Hence assimilation during this phase of growth consists predominantly of the synthesis of proteins.

In the vacuolating, dividing cells it is probable that proportionately more carbohydrates are assimilated than in the cells of the promeristem since during this phase of growth considerably more extension and thickening of the walls occurs than in the promeristem.

Utilization of water in hydration of protoplasm and cell walls, and to a limited degree in vacuolation, also occurs in meristematic regions.

Certain mineral elements in addition to those which are constituents of proteins are also required if the activity of meristematic cells is to be maintained. The more important of these are potassium, iron, boron, manganese, magnesium, and calcium. Several of these elements are believed to act in a regulatory or catalytic capacity in meristematic cells.

During mitosis, therefore, a constant translocation of water, soluble foods, and certain mineral elements in the form of soluble compounds is in progress towards the region of dividing cells. Since the vascular system terminates some distance below the region of the stem in which cell division is occurring,

a question arises regarding the mechanism by which water and solutes, and particularly the latter, are transported into the dividing cells. The rate of movement of solutes towards meristems is usually too rapid to be accounted for by simple diffusion. It is possible that such cells possess the capacity of accumulating solutes or at least of accelerating the rate at which they are translocated as a result of metabolic activity. At least part of the translocation of water and solutes through this zone of the stem axis may occur through the sap-filled intercellular spaces.

Regions of dividing cells are invariably centers of intensive respiratory as well as assimilatory activity and considerable quantities of carbohydrates are oxidized by such cells. Cell for cell the respiratory rate in meristematic regions is higher than in fully matured tissues. Dividing cells undoubtedly utilize energy in many different ways, a number of which have already been listed in Chap. XXIX.

2. *The Cell Enlargement Phase.*—Some cell enlargement usually occurs preceding, during, and immediately following cell division, but usually the increase in the size of the cells during this phase of growth is small compared with that occurring subsequently. Enlargement of cells usually occurs in some tissue zones while cell division is still in progress in others. In many stem tips enlargement and even maturation of the pith and vascular tissues may be occurring while the cells from which the other tissues develop are still in the dividing condition.

While enlargement of cells usually ensues immediately after cell division this phase of growth is not always completed without interruption. In the formation of the buds of woody plants, for example, cell division is followed by only a very limited increase in the size of the cells and the subsequent phases in the growth of the enclosed stem tip are not resumed until the bud opens.

Many of the cells produced by an apical stem meristem enlarge principally in a direction parallel to the axis of the stem, hence this phase of growth is often called *cell elongation.* Both the continued formation of new cells by division and their subsequent elongation result in projecting the stem tip forward along its own axis, which is one of the most obvious manifestations of apical growth. Cell division is generally restricted to the uppermost internodes, but the zone of cell elongation often extends over a long series of internodes. The rate of elongation becomes progressively slower, however, with increasing distance of the internode from the stem tip. The elongation region back of a stem tip is sometimes as much as 10 cm. in length, and in twining plants even longer.

Increase in the volume of all cells does not occur equally; neither do they

all enlarge symmetrically along all axes. Hence cells of very diverse sizes and configurations arise in plant tissues.

The enlargement of plant cells involves primarily an increase—often many fold—in the volume of the vacuoles, and an areal extension of the cell walls. Some increase in the thickness of the cell walls also often occurs during this phase of growth. During the enlargement phase of growth the cell sap disappears from the intercellular spaces and they become filled with air.

During this phase of growth there is apparently little or no increase in the quantity of protoplasm within the cell. As the cells increase in size there is an influx of water into the enlarging vacuoles. Water is also utilized in the hydration of the additional cell wall material which is constructed during cell enlargement. Relatively large quantities of water therefore become integral parts of the cell system during this phase of growth. As the cell increases in size the cytoplasm gradually becomes attenuated into a thin layer which lines the inside of the cell wall, against which it is held by the pressure of the water in the vacuole.

Assimilation of carbohydrate foods continues during cell enlargement because of the continued extension and thickening of the cell walls which occurs during this phase of growth. Cellulose and pectic compounds are the principal cell wall constituents synthesized from carbohydrates. Since little or no additional protoplasm is built up during this phase of growth the consumption of proteinaceous foods during cell enlargement is usually small. It is noteworthy that dividing cells assimilate both carbohydrate and proteinaceous foods, while enlarging cells assimilate almost solely carbohydrates.

The zone of cell enlargement is a region of relatively high respiratory activity. Cell for cell the rate of respiration in regions of cell enlargement is probably little if any less than in meristematic regions.

Rapid translocation of both water and solutes is continuously in progress towards any region of enlarging cells. Like dividing cells, enlarging cells may possess the capacity of accelerating the translocation of solutes into them as a result of the expenditure of energy.

Recent investigations indicate that the areal extension of plant cell walls can occur only in the presence of hormone-like substances called "auxins" (Chap. XXXII).

Two main views regarding the mechanism of cell enlargement have been advanced. One of these holds that the cell wall must first be subjected to elastic (reversible) or plastic (irreversible) stretching as a result of a turgor pressure developed by the cell sap. While in the stretched condition it is assumed that the material substance of the wall is increased either by the intercalation of additional molecules in the wall (*intussusception*), or by

the deposition of additional molecules on the cell wall layers already present (*apposition*). Plastic extension of a wall alone would also result in an increase in its area, but if this occurs unaccompanied by the incorporation of new material the wall necessarily becomes thinner.

A second hypothesis holds that active growth of the cell wall is the primary step in cell enlargement. Growth of the wall is believed to result from the intercalation of additional molecules between those already present. Entrance of water into the cell is considered to be a result of the increase in the volume of the cell rather than its cause. Ursprung and Blum's (1918) finding that while the diffusion pressure deficit of dividing cells is relatively high, due to the appreciable concentration of solutes present, their turgor pressure is low, is considered to be evidence in support of this hypothesis. Continued areal extension of the wall would tend to keep the wall pressure and hence the turgor pressure of the cell at a low value.

3. *The Maturation Phase.*—Size differentiation of cells is largely accomplished during the enlargement phase of growth, but in some types of cells continues during the maturation phase. During the enlargement phase the cells from which certain tissues develop enlarge or elongate much more than those from which other tissues develop. The cells of various tissues differ, however, not only in spatial dimensions, but also in various structural features most of which develop during the maturation phase of growth. Maturation of cells begins earlier in some tissues than in others so that the zones of cell enlargement and cell maturation usually overlap for some distance along the axis of a growing stem tip. Elements of the protoxylem and protophloem often can be distinguished in levels of the growing stem tip in which most of the other cells are still in the enlargement stage. Pith cells also usually become fully matured earlier in the ontogeny of a stem tip than many other tissues.

The cells which develop into the pith and certain other tissues do not elongate greatly along the axis of growth, although their elongation in this direction is greater than radially. Others elongate greatly along the axis of growth and only slightly in other directions, becoming fibers, tracheids, vessel elements, sieve tubes, etc. depending in part upon their position in the organ. Maturation of the cell walls ensues at about the time that cell enlargement ceases. During maturation the walls of practically all cells thicken, although usually not uniformly. The walls of many types of cells and tissue elements become pitted, while in others characteristic structural features are developed, the most striking of which are the spiral and other thickenings of the walls of the protoxylem vessels.

Chemical differentiation of the cell walls also occurs during the matura-

tion phase of growth. The walls of some cells, such as those of the pith, the living cells of the phloem, and most of the cells in the cortex, retain their original cellulose-pectic composition indefinitely. The walls of other cells, such as those of most of the xylem tissues, become lignified. Similarly suberin lamellae develop in the walls of cork cells during maturation.

During maturation cells fall into two classes, those which retain their protoplasmic contents more or less indefinitely, and those in which the protoplasm distintegrates during the process of maturation. In general the protoplasm soon disappears from those cells in which the walls become lignified or develop suberin lamellae, while cells in which such modifications of the wall do not occur retain their protoplasm in an unimpaired condition for a much longer period. Further structural differentiation of walls of cells in which the protoplast dies, such as vessels, tracheids, and fibers, occurs only by such purely physioco-chemical activities as may continue in them, or under the influence of the activities of adjacent living cells. The changes occurring in the heartwood of trees, for example, are a result of such processes.

Disintegration of certain parts of cells may also occur during the maturation stage of growth. A familiar example of this is the disappearance of the cross walls between xylem elements in the formation of vessels.

Assimilation of carbohydrates continues during all types of cell maturation which involve thickening of the cell walls, but practically no proteinaceous foods are assimilated during this phase of growth. In general the respiratory activity of mature cells is distinctly lower than that of dividing or enlarging cells.

The end result of the maturation stage of primary growth is the construction of the tissues of the growing axis according to a pattern which is more or less characteristic for each species. Cells become observably differentiated into epidermal cells, cortical cells, sieve tubes, cambium, vessels, tracheids, pith cells, etc. according to their position in the tissue mass.

The physiological aspects of growth at root tips are fundamentally the same as for growth at stem tips, but there are some important morphological differences between the two. Growth of root tips has already been discussed in some detail in relation to the problem of the absorption of water (Chap. XVI), hence at this point we will merely summarize the principal differences in growth in these two types of apical meristems: (1) The apical meristems of stems are truly terminal while those of roots are located, with rare exceptions, between the root cap and the zone of cell enlargement. (2) The region of cell elongation is usually much shorter (seldom more than 1 mm. in length) than the corresponding region in stem tips. For this reason the entire growth

process is telescoped in root tips as compared with stem tips, the three stages following each other in a more rapid succession. (3) Lateral organs (leaves, floral parts, and side branches) of stems arise from peripheral meristems; those of roots (branch roots) from deeply buried meristematic regions (usually from the pericycle). (4) Lateral organs as a rule arise only in the meristematic region of stem tips, but in roots usually develop well back in the region of cell maturation. As an end result of the growth process in roots not only is the gross morphology of the root determined but its tissues are constructed according to a pattern which is more or less characteristic for each species.

The Rôle of the Cambium in Growth.—The cambium, which is responsible for most of the *secondary growth* (*i.e.* formation of secondary tissues) of plants, is a uniseriate layer of cells which is invariably located between the xylem and phloem. In most plants in which it occurs the cambium is present as an almost continuous sheath of cells extending from just back of every root tip to just below every stem apex. This continuous lamina of cambium is broken, in most species, only at the so-called *leaf gaps* and *branch gaps* (Fig. 119), which occur just above the strands of vascular tissue which lead to the leaves and stems. Usually, however, even such gaps are found only in young parts of the axis, as they are gradually bridged by extensions of the cambium layer as the stem grows older. True cambiums are found only in the dicots and gymnosperms, although some monocots increase in diameter by means of the so-called "secondary cambiums" which will not be considered in this discussion.

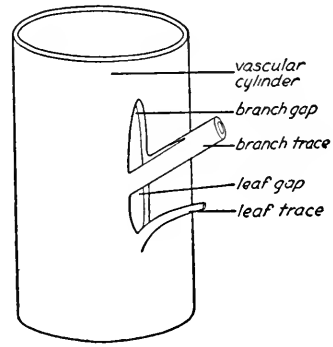

FIG. 119. Diagram of a portion of the vascular cylinder of a stem illustrating leaf and branch gaps. Redrawn from Eames and MacDaniels (1925).

Structurally cambium cells are of two distinct types. The vascular ray initials from which the vascular rays develop are more or less isodiametric. The vertically elongated elements of the xylem and phloem develop from a second and more abundant type of cambium initial. As viewed in cross section the tangential width of cambium cells of this type is usually several times as great as their radial width. The length of such a cambium cell usually exceeds even the greatest of its cross-sectional dimensions by many times (Fig. 120). The cambium initials of a tulip tree, for example, are about 600 μ long, 25 μ in tangential width, and 8 μ in radial width. Much longer cambium cells have been reported in the stems of some conifers. In the

white pine they may attain lengths up to 4000 μ. Although differing structurally from the cells of other meristems, physiologically the properties of cambium initials are essentially similar to those of other meristematic tissues. Cambium cells are vacuolated and often prominently so (Bailey, 1930). They often show protoplasmic streaming.

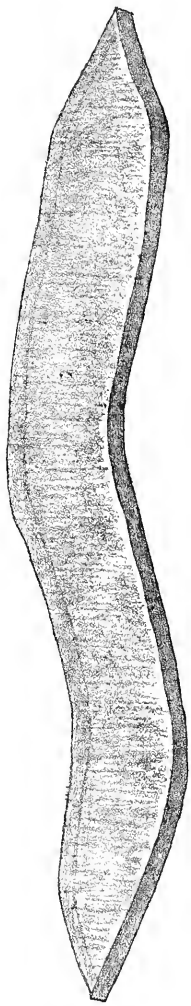

FIG. 120. Perspective view of typical fusiform cambium cell.

The cambium usually becomes active in producing new cells before primary growth has entirely ceased in all of the tissues at the corresponding level of the stem.

Successive radial divisions of the cells of the cambium layer result in the development of *secondary xylem* on its inner face and *secondary phloem* on its outer face and cause increase in the diameter of the axis. Increase in length of the vascular rays also occurs as a result of cambial activity. The secondary xylem and secondary phloem lie between the *primary xylem* and *primary phloem* as the conductive tissues produced during primary growth are termed. All increase in the diameter of stems resulting from cambial activity is due to the production of additional layers of phloem and xylem within the body of the stem. In annual plants or in perennial species in which the stems die down to the ground at the end of each growing season secondary growth of the cambium never continues beyond the current season. In species with woody stems new layers of both xylem and phloem are developed during each period of cambial activity, so that such species exhibit an annual increase in diameter. In some woody perennials the cambium may continue to develop secondary tissues for hundreds of years, cambial activity being resumed periodically with the advent of each growing season. Since in such species the older phloem is first converted into bark and is eventually sloughed off, the bulk of the structure of all older stems and roots is composed of secondary xylem.

Cells originating from the cambium pass through the same three morphological stages of growth as do those developed from apical meristems. Formation of a new cell in the xylem is initiated by division of one of the cells of the cambium layer, the new cell wall developing midway of the cell in the tangential plane (Fig. 55). The outer one of the two daughter cells remains a cambium cell, but the inner one enlarges,

usually in length as well as in cross-sectional area, and generally develops directly into one of the xylem elements. Often, however, the inner of the two cambium derivatives may divide one or more times before maturation of the cells ensues. This usually happens in the formation of wood parenchyma cells during which the xylem mother cell is cut into a vertical series of cells by transverse divisions. Tracheids, fibers, vessels, wood parenchyma and wood ray cells are developed in the xylem from the cambial derivatives (Chap. XV).

A new phloem cell is initiated in a similar manner from the outer of the two daughter cells after division of a cambium cell (Fig. 55). These outer cells may develop directly into mature phloem elements, or may first divide before maturation ensues. In general, division of the phloem mother cells before maturation appears to be of more frequent occurrence than division of the corresponding xylem mother cells. Maturation of the cambial derivatives formed on the outer face of the cambium results in the development of sieve tubes, companion cells, phloem parenchyma, phloem ray cells, and phloem fiber cells (Chap. XXVIII).

Continuation of secondary growth from the cambium results during each growing season in the development of a zone of secondary xylem cells inside of the cambium, and a zone of secondary phloem exterior to it. The enlargement of the xylem cells produced from the cambium initials results in outward movement of the cambium and all of the cells lying outside of this layer, necessarily resulting in an increase in the girth of the cambium cylinder. Enlargement of the immature phloem cells developed from the cambium, on the other hand, results in outward movement only of the phloem and tissues external to it. Generally several times as many new xylem elements as phloem elements are produced by the cambium during a period of growth activity.

Division of the cambium cells results not only in the formation of secondary phloem and xylem, but also, as the stem grows in diameter, in an increase in the girth of the cambium layer. Some increase in the circumference of the cambium cylinder results from a lengthening of the cambium initials along their tangential axis as seen in cross section, but mostly this is brought about by an increase in the number of cells around the cylinder as the stem grows older. According to Bailey (1923) two principal methods by which this occurs can be recognized. New cambium cells may result from radial divisions of older cambium initials (Fig. 121, *A*) or by "pseudo-transverse" divisions followed by a sliding of the derivatives past each other (Fig. 121, *B*). Each of these methods of multiplication of cambium cells is characteristic of certain species.

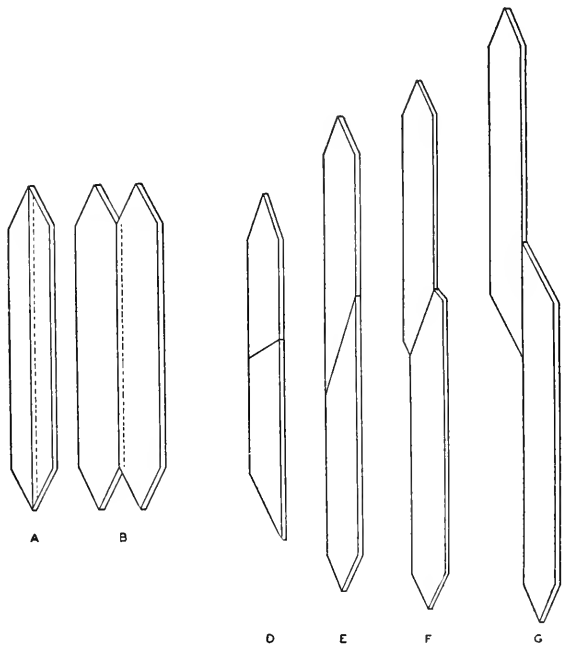

FIG. 121. Diagram illustrating two methods by which cambium cells divide resulting in the formation of additional cambium cells. (*A*) and (*B*) two stages in the radial division of a cambium cell. (*D*) to (*G*) four stages in the "pseudo-transverse" division of a cambium cell followed by a sliding of the two derivative cambium cells past each other.

Development of Lateral Organs.—Practically all of the lateral organs borne on stem and root axes develop during primary growth. Leaves originate from the *leaf primordia* which are small protuberances which develop laterally at more or less regular intervals from the apical meristem (Fig. 117). The point on a stem at which a leaf or leaves develops is called a *node;* the intervening stem segments between the nodes are called *internodes.* The histogenic development of a leaf from its primordium does not follow the same course in all species (Foster, 1936) although there are many points of similarity in the development of most kinds of leaves. As an example we may consider the development of the tobacco leaf (Avery, 1933). The apical leaf meristem in this species consists of a single cell which continues to produce new cells until the leaf is 2-3 mm. long. Until the leaf is about 0.6 mm. long it consists of only a midrib primordium. The lamina originates from two rows of subepidermal meristematic cells, one on each side of the

midrib primordium. Subsequent divisions, enlargement and maturation of these cells result in the development of all of the mesophyll tissues, including the lateral veins. The epidermis increases in area as a result of continued division and enlargement of the epidermal cells. Cell divisions cease first in the epidermis, followed in order by the middle and lower mesophyll, and the palisade layers. The tissues of the lateral veins may continue to develop long after cell division has stopped in other parts of the leaf.

Although cessation of cell division occurs first in the epidermis, enlargement of the cells in this layer continues longer than in any other tissue of the leaf. This results in pulling the cells of the lower layers of the mesophyll apart and in the development of their typical spongy condition. For a similar reason there may also be a limited development of intercellular spaces in the palisade layers. Intercellular spaces do not develop markedly until the leaf has attained one-fourth to one-third its final size.

In the axil of each embryonic leaf a mound-like lateral meristem develops (Fig. 117). This becomes a lateral bud which is essentially a rudimentary side branch. In many woody plants terminal buds also form annually at the tip of each stem axis. Growth from these buds is usually resumed with the advent of the next growing season. In temperate zone woody plants these rudimentary stem tips are encased in bud scales, which are shed only when and if growth of the stem tip is resumed. The buds of most herbaceous plants are devoid of bud scales. Most plants produce a great many more axillary buds than ever develop into lateral branches. Whether or not one of the rudimentary stem tips which is the essential part of a bud will ever resume growth depends in part on environmental factors, and in part on internal conditions.

Some of the stem meristems on most species of plants become differentiated into inflorescences. In some species inflorescences develop from terminal buds, in others from lateral buds. Some buds produce vegetative parts only, some both vegetative and flower parts, and some only flower parts. These are usually designated as *vegetative buds, mixed buds,* and *flower buds,* respectively. A flower bud is essentially a more or less abbreviated stem tip which becomes differentiated into either a single flower or an inflorescence.

The meristematic activity of flower-bearing shoots does not usually cease with the production of flowers, although elongation of the shoot often terminates with blooming. If pollination and fertilization occur (and sometimes in the absence of these processes) the ovulary of the flower and often other parts as well develop into a fruit, while the enclosed ovules and their contents mature into seeds. The formation of these organs involves the same three morphological phases of growth as the production of vegetative organs.

Branch roots develop on the main root axis, as previously described in Chap. XVII, from the pericycle (usually) which remains in an essentially meristematic condition for some distance back of the growing point of the root. Such branch roots progress through the soil by elongation of the cells produced as a result of cell division in the apical meristem, and usually produce branch roots in turn.

Measures or Indices of Growth.—It is frequently desirable to give some sort of a quantitative expression to the amount of growth which is accomplished by a plant or a group of plants during a given period of time. The principal indices which have been employed for this purpose are (1) increase in the length of the stem, root, or other organ of the plant, (2) increase in the area of the leaves, (3) increase in the diameter of the stem (or other organ), (4) increase in volume (especially of fruits), (5) dry weight increment, and (6) fresh weight increment.

All of these indices have at least a limited value as measures of growth, especially from various practical standpoints. Determinations of the height and diameter growth of forest trees, for example, are standard forestry practices as indices of the productivity of forests, and have considerable practical value for such purposes. Similarly the number of tons of dry hay or the fresh weight of cabbage or spinach produced per acre would usually be an adequate measure of growth to the mind of the practical farmer.

Each of the indices listed above, however, measures only certain quantitative phases of growth. A yardstick can measure only length, a balance only weight, but growth phenomena generally involve not only such quantitative changes as expansion in length and girth and increase in weight, but qualitative aspects as well. How, for example, could the relative development of the vegetative and reproductive phases of growth be expressed in terms of any of the units listed above? Yet qualitative differences in growth are often of as great or greater scientific significance or practical importance as quantitative differences. The floriculturist is not primarily interested in the pounds of plant substance produced nor the height to which his plants grow, if they bear flowers which will be attractive to his customers. Likewise the orchardist is much more interested in the development of the fruits on his trees, than in the increase in the height or weight of their vegetative organs. Evidence of this difficulty in giving adequate expression to the results of growth phenomena is seen in the common expedient of investigators in relying upon photographs as a means of recording the results of their experiments upon the growth of plants.

Growth Curves.—The generalized aspects of the rate of growth of plants can often be given definite expression in graphical language. Such

"growth curves" are usually plotted in terms of rate of growth or total growth increment against time. The rate of growth or growth increment may be expressed in terms of any of the commonly accepted indices of growth—elongation, enlargement, volume increase, dry weight increase, or fresh weight increase. Since these indices are principally or entirely measures of the cell division and cell enlargement phases of growth, curves plotted in terms of such units represent only the quantitative phases of growth.

Measurements of the rate of growth (increase in length per day) and total growth increment (cumulative length at time of measurement) for the hypocotyls of the muskmelon (*Cucumis melo*) are shown in Table 56.

TABLE 56—RATE OF GROWTH AND GROWTH INCREMENT OF MUSKMELON COTYLEDONS AT 20° C. (DATA OF EDWARDS, PEARL, AND GOULD, 1934)

Days after planting	Growth per day (mm.)	Total growth increment (mm.)
3	2.4	2.4
4	5.7	8.1
5	10.8	18.9
6	19.0	37.9
7	26.0	63.9
8	30.7	94.6
9	28.3	122.9
10	22.0	144.9
11	12.4	157.3
12	6.8	164.1
13	3.3	167.4
14	1.2	168.6
15	1.1	169.9
16	0.6	170.3
17	0.4	170.7

If these data for the *rate* of elongation be plotted against time a curve such as that shown in Fig. 122, *A* will result. Similar curves will result if the rate of increase in size of any plant cell or organ be plotted against time. The general trend of all such curves indicates that elongation is at first slow, then steadily increases until a maximum rate is attained, after which a slow but steady diminution in rate sets in, until eventually increase in the size of the cells ceases entirely. Similar curves will usually result if the rate of increase in fresh weight or rate of increase in dry weight is taken as a quantitative index of growth instead of the rate of enlargement. Every plant cell and hence every coordinated group of cells undergoes such a cyclic change in the rate of enlargement during the growth period. The time period

corresponding to this cyclic variation in growth is termed the *grand period of growth*. That this is a universal pattern of growth behavior is shown by the fact that it is exhibited by such diverse types of growth phenomena as the rate of elongation of segments of the root axis, the rate of elongation of segments of the stem axis, the expansion in area of leaves, the increase in weight

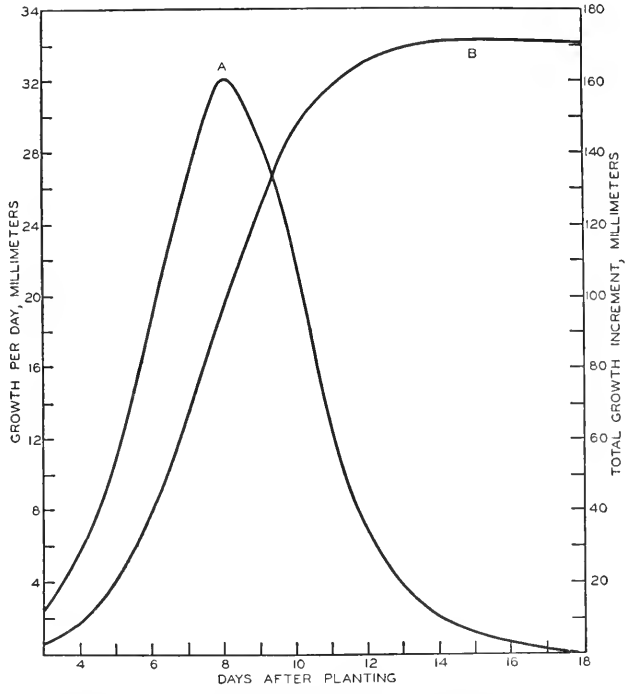

FIG. 122. Growth curves of muskmelon hypocotyls. (*A*) curve of daily increment of elongation. (*B*) curve of total increment of elongation. Data of Edwards, *et al.* (1934).

of fruits, the growth of annual plants expressed in terms of dry weight increment, and even the growth (*i.e.* increase in population) of micro-organisms.

Although environmental factors may influence the length of time required for completion of the grand period of growth, or in extreme cases may cause complete cessation of growth thus causing interruptions in the cycle, usually the general trend of the grand curve of growth is immutable, indicating that it is primarily controlled by internal factors. This does not mean that the magnitude of the growth in a given plant may not vary greatly in ac-

cordance with prevailing environmental conditions, but simply that the relative rate of growth at any time during the growth period normally bears a definite relation to the relative growth increment which has already occurred and which may be expected to occur subsequently. A maize plant, for example, which has developed under adverse environmental conditions may attain only half the stature of a similar plant which has completed its growth under more favorable circumstances, yet if curves representing the growth rate of the two plants be plotted both will assume the characteristic shape exhibited by Fig. 122, *A*, although the actual magnitude of the values on the two curves will be very different.

If the total increment of growth instead of the rate of growth be plotted against time the resulting curve will assume a typical sigmoid shape (Fig. 122, *B*). Any growth phenomenon which is represented by a curve of the type shown in Fig. 122, *A*, when plotted in terms of growth rates, will yield a sigmoid curve when depicted graphically in terms of growth increments. Hence such sigmoid curves of growth are characteristic of a wide variety of growth phenomena.

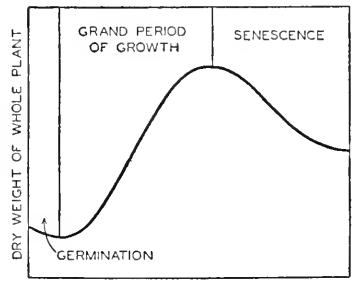

FIG. 123. Generalized growth curve for the life cycle of an annual plant.

Equations can be derived which indicate in mathematical terms growth relations such as those depicted graphically in Fig. 122, *B*. A number of attempts have been made to attach some special significance to such mathematical formulations of the rate of growth. It has been considered, for example, that the mathematical expression representing a sigmoid growth curve is identical with the equation of a monomolecular autocatalytic reaction (Robertson, 1923; Reed, 1920) and by others as representing the same type of equation as that expressing the increase in a sum of money at compound interest (Blackman, 1919). All such concepts are, however, of doubtful or limited validity, at least as applied to the higher plants, and will receive no further attention in this discussion.

A growth curve plotted in terms of the increment in dry weight for the entire life history of an annual plant will assume the generalized form indicated in Fig. 123. Such a curve can most readily be pictured as representing the growth increment in terms of dry weight of a crop plant such as maize. During the germination stage there is usually a slight loss of dry weight due to respiration at a time when photosynthesis has not yet attained any appreciable rate. Following this the curve swings into the characteristic sigmoid

shape representing the grand period of growth. During the earlier stages of this period the leaf area of the plant and hence its photosynthetic capacity increase at an accelerated rate. Correlated with this is a progressive acceleration in the rate of increase in dry weight. Some of the increase in dry weight is also due to the absorption of ions from the soil but proportionately this is always very small. Midway of the grand period a reversal in trend becomes apparent. From this point on there is a gradual deceleration in growth increment until increase in dry weight ceases entirely. As the plants mature the photosynthetic efficiency of the leaves decreases, and in some species leaves may actually be shed during this period. Reproductive development usually occurs during this period of deceleration in rate of increase in dry weight. Most of the foods synthesized are diverted into the developing fruits and seeds, consequently vegetative growth is suppressed and new leaves are not produced with sufficient rapidity to compensate for the loss of photosynthetic activity in the older leaves.

Finally the plant passes into a state of senescence during which the respiration rate usually exceeds the rate of photosynthesis. Hence during this period of decline the plant loses dry weight. The later stages in the ripening of fruits and seeds often occur during this period of senescence; in fact the high respiratory activity of such organs often accounts for much of the loss of dry weight during this period. Abscission of leaves and fruits may also be responsible for a considerable proportion of the loss in dry weight during senescence in some species.

The growth cycle of an annual plant is terminated by its death at which time it still usually retains a large proportion of the substance produced as a result of its synthetic activity. Much of this is in the form of the cellular structure of the plant body, but in many plants a considerable proportion of the accumulated dry matter represents assimilated and accumulated foods in the ripened fruits and seeds, some or all of which are retained in many annual species until after the death of the vegetative organs.

Rates of Growth.—The absolute growth rates recorded in the botanical literature are mostly expressed in terms of increase of height of stems, although some figures are available in terms of dry weight increment and other units.

The rate of height growth varies enormously with different species of plants, and with the same species under different environmental conditions. Only a few examples of the most rapid known rates of height growth will be cited. Young bamboo shoots occasionally grow as rapidly as two feet in twenty-four hours. When a flowering stalk is produced by the century plant it often elongates as much as six inches during the course of a single day.

Under favorable growing conditions corn plants sometimes add visibly to their stature during a single night.

The rates of elongation of the fastest growing stems are just a shade too slow for detection with the naked eye. By observing rapidly growing stem tips under a horizontally placed microscope the externally visible aspects of growth can often be observed and measured directly.

The rate of growth of stem or root tips can also be measured by the very simple method of marking the organ with short horizontal lines spaced equidistantly. The marks are generally made with India ink applied with a fine brush. The rate of elongation is determined by observing the position of the marks after the lapse of a definite period of time. A similar method can be used for measuring the rate of increase in the area of a leaf by ruling a cross-sectional pattern of lines on the leaf. Such methods are of value in indicating in what part of the organ enlargement is occurring most rapidly.

A number of different types of instruments have been devised for the automatic measurement of the rate of height or diameter growth in plants.

Accumulation of Foods.—Although the simple carbohydrates synthesized in photosynthesis may undergo many transformations the sum total of the food available to a green plant can never exceed the amount produced in photosynthesis. A large proportion of the photosynthate is normally consumed in the processes of assimilation and respiration. Any surplus which remains accumulates in one or more tissues or organs of the plant. Accumulation of foods, however, does not occur continuously. For considerable periods in the life cycle of most species not only is no accumulation of food occurring, but a more or less rapid consumption of food reserves is in progress. In woody plants during the dormant season slow utilization of food in the processes of respiration and assimilation continues. When growth is resumed in the meristematic tissues of such species in the spring, there is always a considerable drain on the supply of foods stored in the plants since much of this growth is accomplished before the photosynthetic rate is rapid enough to compensate for the necessarily speedy utilization of food which occurs. Similarly the sprouting of bulbs, corms, tubers, rhizomes, etc. always occurs at the expense of the stored foods in such organs. The same is true of seeds when germination occurs. Much of the food which accumulates in plants during periods when photosynthesis exceeds the food-consuming processes is utilized by the plant sooner or later in its life history.

The organs in which most accumulation or "storage" of food occurs are different in different species. In annuals food storage occurs predominantly in the seeds. Foods also accumulate in the seeds of most biennial and perennial

species. During the process of germination the embryo uses food that was made by the preceding sporophyte generation.

Most of the accumulation of food in typical biennials such as beet, carrot, parsnip, turnip, etc. occurs in fleshy roots or root-like structures. This accumulation of food by biennial species occurs mostly during their first season's growth. During the second season most of the accumulated food is utilized in the production of flowers, fruits, and seeds so by the end of their life cycle the vegetative parts of such plants contain relatively little food.

In perennial species considerable storage of food often occurs in seeds and fruits but the principal organs of food accumulation in many species which live for a number of years are the stems and roots. In woody species the pith, cortex, vascular rays, and wood parenchyma are the stem and root tissues in which most of the accumulation of surplus foods occurs. Modified stems such as rhizomes (iris, many ferns, Solomon's seal, etc.) tubers (potato, Jerusalem artichoke, etc.) corms (crocus, gladiolus, jack-in-the-pulpit, etc.), and bulbs (onion, tulip, hyacinth, etc.) are almost invariably regions of food storage in species which possess such organs.

The great bulk of all foods which accumulate in plants can be classified into the familiar categories of carbohydrates, fats and proteins.

The principal storage carbohydrates are starch, sucrose, "hemicelluloses" and inulin (Chap. XXII). Accumulation of oils (fats) in abundance occurs most commonly in seeds, although such compounds are stored in at least small quantities in the cells of many tissues. Proteins, like fats, accumulate principally in seeds.

The cells of "storage" tissues are not merely passive reservoirs in which excess foods pile up. Foods move into the cells in which they accumulate only in the soluble form, yet with few exceptions, sucrose being the only important one, the foods amassed in storage cells are converted into an insoluble form. Upon translocation into storage cells, glucose is converted into starch, amino acids into proteins, fructose into inulin, fatty acids and glycerol into fats, etc. All of these chemical transformations are condensation reactions which are catalyzed by enzymes occurring in the living cells in which the foods accumulate.

Conversely, stored foods cannot be utilized by any part of a plant until they have first been digested into soluble forms as a result of enzymatic activity. Until such a transformation has occurred they cannot be translocated out of the cells in which they are situated into the cells in which they are utilized in assimilation or respiration.

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CHAPTER XXXII

GROWTH HORMONES

Among the substances which markedly influence the reactions and metabolism of the animal body are those internally produced compounds called *hormones*. Many of the hormones of the higher animals are secreted by the ductless glands; examples are adrenalin, thyroxin, and insulin. Within the last decade or two convincing evidence has accumulated that hormone-like substances also occur in plants. These compounds are called *growth substances, hormones, growth hormones,* or *phytohormones*. Like animal hormones plant hormones quite commonly affect parts of the organism other than those in which they are produced. It is a characteristic of both plant and animal hormones that they usually exert their physiological effects while present in minute concentrations; it is principally on this basis that they differ from compounds ordinarily classified as foods.

Relation of Auxins to Growth of the Oat Coleoptile.—The *auxins* are the best known and most comprehensively studied group of plant hormones. Their action has been most clearly demonstrated in the leaf sheath or *coleoptile* of the oat plant (*Avena sativa*). This is a tubular, leaf-like structure, closed at the top, which is the first organ of the plant to emerge from the soil. Similar coleoptiles are produced during the germination of seeds of other members of the grass family. The coleoptile encloses the first leaf, and is eventually pierced at the tip as a result of the growth of this leaf, soon after which all growth in length of the coleoptile ceases. Oat coleoptiles are approximately 1.5 mm. in diameter, and when illuminated seldom attain a length of more than 2 cm. In the dark they may attain heights ranging up to 6 cm. Cell divisions cease relatively early in the life history of an oat coleoptile, and during approximately the last three-fourths of its growth period all increase in its length is due to elongation of its constituent cells (Avery and Burkholder, 1936).

If the tip of a coleoptile is removed by a clean cut made several millimeters below the apex, the rate of growth of the stump is immediately retarded (Söding, 1925). If, however, the cut-off tip of the coleoptile or a similar tip from another coleoptile if affixed on the stump, its growth will be resumed,

and may nearly regain the original rate (Fig. 124). Retipping the coleoptile with a short segment cut out of another coleoptile somewhat below the apex results in little or no increase in growth rate. Such experiments indicate that the growth of a coleoptile, which occurs in the more basal regions, is maintained only under the influence of some sort of a "stimulus" originating in the tip, whence it is transmitted basipetally (apex to base) through the coleoptile. Furthermore, since at the age in their development at which coleoptiles are used in such experiments cell divisions have ceased, this influence is exerted on the enlargement phase of growth.

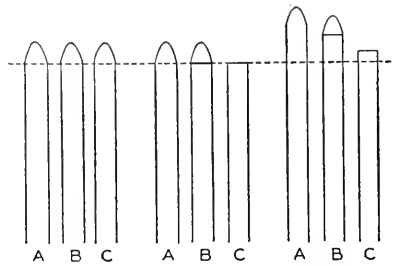

FIG. 124. Effect of removal of tip on elongation of oat coleoptile. (*A*) check, (*B*) tip severed and replaced, (*C*) tip removed. The effect of treatment is shown by differences in increase in length of coleoptiles at right.

Went (1928, 1935) placed the cut-off tips of oat coleoptiles on a thin layer of 3 per cent agar and after one hour removed them and cut the agar into a number of equal-sized small blocks (Fig. 125). If one of these blocks was placed upon the stump of a decapitated coleoptile, the rate of elongation was accelerated just as if the stump had been reheaded with a fresh coleoptile tip. On the other hand, retipping a coleoptile with a block of pure agar had no appreciable accelerating effect on elongation. It seems evident from these results that some substance or substances was transported out of the tip into

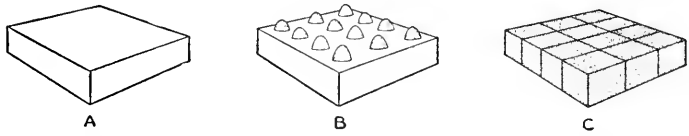

FIG. 125. Diagram illustrating stages in the collection of auxin in agar from coleoptile tips.

the agar block, and subsequently out of the block into the tip of the decapitated stump, whence it was translocated downwards to the elongating region of the coleoptile (Fig. 126). The substances which induce such a response are now classed as auxins.

Many other plant organs such as stems, petioles, flowerstalks, and coleoptiles of other species behave similarly upon removal of the apical region. Elongation is stopped or retarded by such a treatment, but will be resumed

if the excised apex of the organ is carefully relocated on the cut surface of the stump.

Use of the Oat Coleoptile as a Test Plant in the Quantitative Determination of Auxins.—Auxins are now known to be of widespread distribution in plants. They occur in such small quantities, however, that detection of their presence in an organic material by chemical methods is usually difficult and often impossible. Recourse is had, therefore, to sensitive biological tests in order to demonstrate the presence of these substances. The oat coleoptile test is the most commonly used method of determining the relative quantities of auxins present in plant tissues or other materials.

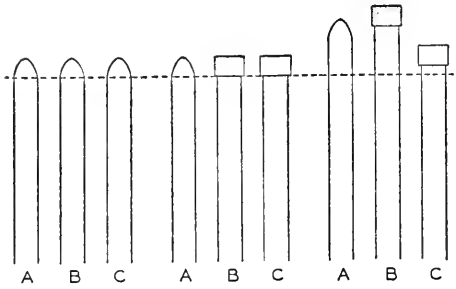

FIG. 126. Effect of agar blocks containing auxin on elongation of oat coleoptile. (*A*) check, (*B*) block containing auxin placed on decapitated tip, (*C*) block of pure agar placed on decapitated tip. The effect of the treatment is shown by relative increase in length of coleoptiles at right.

If an agar block containing auxins from one source or another is affixed one-sidedly on the decapitated stump of an oat coleoptile, elongation is found to be more rapid on the side of the coleoptile below the portion of the tip on which the block is perched, resulting in curvature of the coleoptile (Fig. 127). Translocation of the hormone is almost strictly longitudinal, the elongating cells on the side of the coleoptile covered by the block receiving much more than cells on the opposite side, with a corresponding differential effect on growth. When the block is centered on the decapitated tip, as in the experiment described in the preceding section, all sides of the coleoptile receive approximately equal quantities of auxin, and growth proceeds in a vertical direction.

Furthermore, it has been found that the curvature resulting from the eccentric attachment of agar blocks to the decapitated stumps of oat coleoptiles is proportional, within the range of about 0 to 20 degrees, to the concentration of the auxin in the agar block. This proportionality between hormone concentration and curvature makes possible the use of oat coleoptiles as living test objects in the quantitative estimation of the auxin content of plant tissues, etc. (Went, 1928).

Quantitative measurements of auxins by the oat coleoptile technique must be carried out under carefully standardized conditions. Only a generalized description of the usually employed methods will be given. The oat seedlings

(usually from a genetically uniform variety) are grown in a dark room at a temperature of 25° C. and a relative humidity of 90 per cent. All manipulations are performed under phototropically inactive orange or red light (Chap. XXXVII). The coleoptiles are used when about 2.5 to 4 cm. in length. The extreme tip of the coleoptile is first cut off, and after three hours the topmost 4 mm. of the stump is removed. For reasons which can not be considered in a brief discussion, the coleoptiles are more sensitive when this method of double decapitation is employed than when the tip is cut off in one operation.

The primary leaf, which is enclosed by the coleoptile, is then pulled loose so it will not interfere with the determination by its continued growth. If

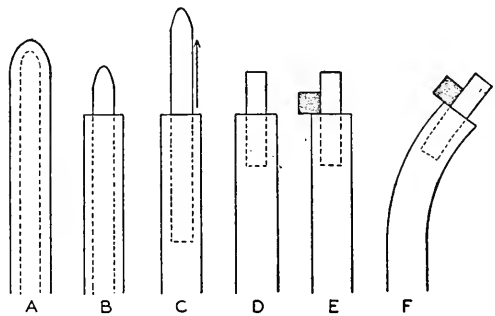

FIG. 127. Diagram of method of determining auxin content of agar block quantitatively. (*A*) intact coleoptile enclosing primary leaf, (*B*) tip of coleoptile removed, (*C*) primary leaf pulled loose so its elongation will not dislocate agar block, (*D*) tip of primary leaf cut off, (*E*) agar block affixed unilaterally to coleoptile tip, (*F*) curvature resulting from movement of auxin into side of coleoptile below agar block. Redrawn from Went (1935).

guttation water exudes at the cut surface it is carefully blotted off. An agar block (2 × 2 × 1 mm. is a commonly used size) containing the substance to be tested is then affixed unilaterally to the cut tip. After a standard length of time (usually 90 min.) the resulting degree of curvature of the coleoptile is determined. This is usually done by obtaining their "shadowgraphs" on bromide paper and measuring the curvature of the shadowgraphs with a suitable protractor. The greater the degree of curvature, within limits, the greater the hormone concentration in the agar block.

Various methods of applying the material to be tested for auxins to decapitated coleoptiles have been employed. Sometimes small plant organs or pieces of plant organs are affixed directly to the cut surface of the coleoptile. More commonly plant tissues are placed in contact with moist 3 per cent

agar into which the hormone will "diffuse." This procedure has already been described for coleoptile tips. The tissue is generally left in contact with the agar for about two hours. The agar is then cut into blocks of standard size which are affixed unilaterally to the decapitated coleoptiles. The effect of pure chemicals, extracts, etc. can be tested by first dispersing them in agar, and then, after solidification, determining the influence of standard sized blocks of this agar on curvature of the coleoptiles. Or agar blocks can be first soaked in a solution of the substances and then used in the coleoptile test for growth hormones.

Another method which has been widely used in testing for growth hormones is to dissolve the substance in lanolin (wool-fat) and apply the resulting paste directly to the coleoptile or other plant organ.

The Occurrence and Synthesis of Auxins in the Plant.—The occurrence of auxins in plants not only can be demonstrated by the oat coleoptile and other biological tests, but their presence can be shown in many materials by direct extraction with chloroform and other solvents (Thimann, 1934). Auxins are apparently universally present in plants, and their occurrence has actually been demonstrated in a wide variety of species. Furthermore, the auxins are non-specific in their action, *i.e.* the same auxin, chemically speaking, which influences growth phenomena in one species, apparently also influences the same phenomena in all other species. Although of wide occurrence in plants the concentration of auxin may vary greatly from one part of a plant to another, and in some tissues which have been tested it has been impossible to demonstrate their presence.

Auxins have also been found in animals, but are not believed to serve any essential rôle in the animal body. It is probable that such auxins have their origin in plant materials consumed by animals.

Auxins are synthetic products of plant metabolism. They seem to be produced principally and perhaps entirely in the apical meristematic tissues, such as buds on growing stems, young leaves, the apical portions of coleoptiles and flowers or inflorescences on growing flower-stalks. The production of auxins seems often to be associated with the synthesis of protoplasm. Although apparently synthesized in certain tissues, auxins may subsequently be distributed to other organs of the plant. In general they are found in the greatest concentrations in those tissues in which they are produced or stored.

There is good evidence that auxin is formed from a precursor and that light is necessary for the synthesis of this precursor, but not for its transformation into the auxin. Temperature is also a factor in auxin synthesis; in the oat coleoptile the optimum is about 25° C. Present evidence indicates that the auxin is apparently synthesized in coleoptile tips from a precursor which

is translocated acropetally (base to apex) through the coleoptile from the seed (Skoog, 1937).

Chemical Nature of the Auxins.—In a brilliant series of investigations beginning in 1931 Kögl and his co-workers have succeeded in isolating from biological sources three chemically pure crystalline substances which give all the reactions of auxins when tested by the oat coleoptile technique. These three substances have been called auxin *a* ($C_{18}H_{32}O_5$), auxin *b* ($C_{18}H_{30}O_4$), and heteroauxin ($C_{10}H_9O_2N$). The chemical names of these substances are auxentriolic, auxenolonic, and indole-3-acetic acid, respectively. All three of these compounds are monobasic acids. Auxin *a* has been prepared in the pure state from human urine, and both auxins *a* and *b* have been isolated from malt and various vegetable oils. Hexeroauxin has been isolated from urine and from certain yeasts and molds. This compound (indole-3-acetic acid) has also been prepared synthetically.

More recently it has been discovered that a number of other compounds also result in curvature of oat coleoptiles when tested by the usual technique and by some investigators all such compounds are classed as auxins. Most of these compounds are not as effective in inducing this response as the three described above, and it has not been shown that any of these compounds occur naturally in plants. The physiological effectiveness of auxins *a* and *b* and heteroauxin is almost unbelievable. It has been calculated that 1 mg. of either auxin *a* or auxin *b*, applied in agar blocks, can cause a curvature of 10 degrees in 50,000,000 oat coleoptiles. Heteroauxin is about half as effective.

The question of which is the naturally occurring auxin in various species of plants has not yet been settled with finality. Present indication are, however, that auxin *a* is the naturally occurring auxin in oats and other higher plants while heteroauxin is of common occurrence in fungi.

The Translocation of Auxins.—The transport of the auxins from one part of a plant to another presents some distinctive and interesting features. If a block of agar containing auxin is affixed to the morphologically upper end of a segment of oat coleoptile, and a block of pure agar to the lower end, auxin will move into and accumulate in the lower block. The final concentration of auxin in the basally attached block may greatly exceed that in the one affixed to the apex (Fig. 128, *A*). If the position of the coleoptile segment between the agar blocks is reversed, *i.e.* if the block containing auxin is affixed to the morphologically basal end no translocation of auxin will occur (Fig. 128, *B*). Translocation of auxin in oat coleoptiles apparently takes place through the parenchyma tissue. The results of such experiments show: (1) that translocation of auxin in the oat coleoptile is *polar, i.e.* occurs only basi-

petally, and (2) that it can occur against a concentration gradient since it accumulates in the lower block.

According to van der Weij (1934) the rate of auxin transport in oat coleoptiles is about 10-12 mm. per hour and is almost entirely independent of temperature. The auxin transporting capacity of the coleoptile (*amount* transmitted per unit time), however, increases rapidly with rise in temperature from 0° C. to about 35-40° C.

A similar basipetal transport of auxins apparently also occurs in many other plant tissues and organs such as the veins of leaves, petioles, hypocotyls, and stems of various species. In many such organs translocation seemingly takes

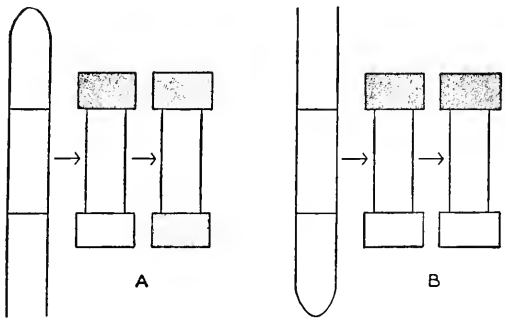

FIG. 128. Diagram to illustrate basipetal movement of auxin. (*A*) Agar block containing auxin attached to apical end of segment of oat coleoptile, (*B*) agar block containing auxin attached to basal end of segment of oat coleoptile. Redrawn from Went (1935).

place only through the vascular bundles, most probably occurring in the phloem.

The mechanism of the polar transport of auxins has been subject to much conjecture and considerable experimentation. Etherization stops the transport of auxin, except insofar as it can be accounted for by diffusion, and destroys its polarity. This indicates that living cells are involved in the process, but tells nothing of the manner in which they operate.

It has been suggested that the polarity in the movement of auxins may be due to differences of electrical potential in the tissues. Apical portions of many plant organs, including coleoptiles, are usually relatively more negative than basal portions (Chap. XXXIV). Since the auxins are acids their active radicals carry a negative charge and would be expected to move towards the more positive basal regions of such organs. It has not, however, been possible to demonstrate any causal relation between the electrical polarity of plant tissues and the direction of auxin transport through them (Clark, 1937).

Not all translocation of growth substance in plants occurs basipetally, however. Hitchcock and Zimmerman (1935, 1938) have shown that upward transport of certain growth substances occurs when they are supplied to the plant from an external source. For example, when relatively high concentrations of certain growth substances were added to the soil, they were absorbed and translocated in an upward direction through the plants rooted therein. It is not certain at the present time, however, to what extent naturally occurring auxins move in an upward direction through plants.

The Rôle of Auxins in Cell Elongation.—The influence of auxins on cell elongation in the oat coleoptile has already been described. Auxins play a similar rôle in the elongation phase of growth in many other plant organs, and it is now generally considered that cell elongation occurs only in the presence of auxins. In the absence of these substances elongation fails to take place and within limits elongation is proportional to the concentration of auxins, providing no other factor necessary for growth is limiting. It appears to be true, however, that auxin frequently is the limiting factor in cell elongation. Increase in concentration beyond the point at which some other factor becomes limiting results in no further elongation (Blackman's principle, Chap. XXI). Relatively high concentrations of auxins, in fact, often exert an inhibiting effect on the elongation of aerial organs. The optimum range of concentrations for cell elongation varies greatly with different tissues.

If the extreme tip of a maize or lupine root is cut off, its rate of elongation increases, although not greatly (Cholodny, 1926). Replacement of the root tip in maize plants results in a retardation in elongation rate as compared with decapitated roots. Furthermore, attachment of coleoptile tips of maize to decapitated root tips of the same plant results in a retardation in the elongation rate of the root tip. These results suggest that the same concentrations of auxins which accelerate elongation in coleoptiles and other aerial organs retard elongation in roots.

This supposition has been confirmed by experiments in which the roots of oat seedlings were immersed in pure solutions of auxins. The growth of the roots was found to be retarded in proportion to the concentration of auxin used. However, when roots which contain either no auxin at all or virtually none are treated with auxin solutions of very low concentration acceleration of growth as compared with similar but untreated roots often results.

The presence of auxin in roots has been demonstrated by chemical as well as by biological tests, and in general it is found to be present in greatest concentration in the root tips. It is not definitely known whether or not intact roots actually synthesize auxins. The evidence at present available indicates

that the auxin present in roots is entirely or largely a result of downward translocation from aerial organs.

The recent suggestion by Thimann (1937) that roots, buds, and stems all react in a comparable way to auxin, their growth being inhibited by relatively high and promoted by relatively low auxin concentrations, is a possible explanation of the contrasting effects of auxins upon elongation in roots and aerial organs (Fig. 129). Elongation of roots is favored only at very low concentrations; at all higher concentrations their growth is checked. Stems and coleoptiles show a similar behavior except that the optimum range of concentrations for elongation is much higher than for roots. The same concentrations of auxins which favor stem elongation result in a retardation of root elongation. The effect of auxins on bud development is considered in Chap. XXXIV; for the moment we need only note that they seem to occupy

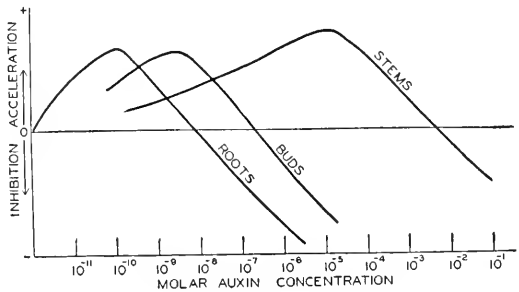

FIG. 129. Relation of auxin concentration to elongation of roots, buds and stems according to Thimann (1937).

an intermediate position between roots and stems with respect to their response to different auxin concentrations. Briefly, therefore, whether auxin will exert an accelerating or an inhibiting effect upon growth seems to depend in part upon its concentration and in part upon the specific tissue involved.

Nothing is known of the mechanism of the inhibiting effects of the auxins beyond the not very helpful suggestion that in relatively high concentrations they may exert a "toxic" effect. The elongation-promoting influence of the auxins, on the other hand, has been the subject of considerable experimental study but as yet very little conclusive evidence of the mechanism of this effect has been forthcoming. A reasonable presumption is that the auxins in some manner influence the intercalation of new molecules during the areal extension of cell walls. Some authorities believe, however, the auxin acts merely in such a manner as to increase the plasticity (irreversible extensibility) of the

wall. Some auxin is actually used up in the growth process, but the amounts consumed are very small. Thimann and Bonner (1933) have calculated that one molecule of auxin brings about the deposition of 300,000 hexose residues in the form of cellulose during the growth of oat coleoptiles.

Effect of Auxins on Root Formation.—It has been known for many years that the presence of buds on a cutting favors development of roots when the basal portion is introduced into a suitable rooting medium. Developing buds are more effective in promoting root formation than quiescent buds. Leaves, especially if young, also often favor the production of roots on cuttings. These observations suggest that root initiation on cuttings is favored by growth substances which are produced in the buds and young leaves and are subsequently translocated to the basal part of the cutting.

The activity of various substances in promoting root formation can be tested by a number of different techniques. Went (1934) has described a quantitative method which consists essentially in immersing the apical portions of cuttings from etiolated pea seedlings in the solution to be tested for 15 hours. The terminal bud is removed and the apex of the stem is split back for 1 or 2 cm. before immersion. Basipetal translocation carries substances favoring root formation to the lower end of the cutting. The basal ends of the cutting are then immersed for seven days in 2 per cent sucrose solution, followed by seven days in water. The number of roots formed is considered to be a measure of the "root-forming capacity" of the substance in the solution.

FIG. 130. Root formation on stem of tomato plant resulting from treatment with lanolin containing 2 per cent alpha naphthalene acetic acid. Photograph from Zimmerman and Wilcoxon (1935).

By the use of this technique and others it has been shown that auxin *b* and heteroauxin are both active in causing root formation. It is now generally considered that at least one of the naturally occurring hormones which favors root formation is identical with one of the auxins, and that one or more hormones is required for the formation of roots, whether they develop on other roots, on stems, or on leaves.

Subsequently a number of other compounds have been found which promote root formation on stems or cuttings. Zimmerman and Wilcoxon

(1935) have shown that a number of substances will promote root formation when applied to stems in lanolin paste or when injected as aqueous solutions (Fig. 130). The most effective of these compounds in root formation were alpha-napthalene acetic acid, and indole butyric acid. Neither of these compounds is known to occur in plants, but both cause curvatures when tested on oat coleoptiles (Avery, *et al.,* 1937).

Several of these compounds, notably indole-3-acetic acid, alpha-naphthalene acetic acid and indole butyric acid, are now being widely used in a practical way for speeding up the rooting of commercially important plants. The usual procedure is to immerse the basal end of the cuttings in an aqueous solution of the substance used (Hitchcock and Zimmerman, 1936). Such treatments not only speed up rooting, but induce the production of an increased number of roots, and cause emergence of roots to take place over a greater area of the stem. Treatment for about 24 hours with a solution containing 4 to 20 mg. per 100 cc. of any of these compounds has been found to be effective on a number of different species.

Root formation is influenced by a complex of factors, all of which must be considered in evaluating the effect of auxin. Carbohydrates and other foods are necessary and evidence is gradually accumulating that certain other hormone-like substances are required. Vitamin B_1 (thiamin), for example, is apparently necessary for root formation (Robbins and Schmidt 1938; Went, *et al.* 1938). Even when auxin is present in adequate quantities production of roots will not occur if one of these other factors is limiting.

Other Effects of Auxins.—The physiological phenomena described in this chapter are not the only ones known to be influenced by auxins. Other important physiological effects of these substances are described in Chapters XXXIV, XXXVI, and XXXVII.

Other Plant Hormones.—Despite their manifold effects it seems clear that the auxins are only one of a number of types of hormones which occur in plants. For example, Haberlandt (1921) showed that if a freshly cut plant tissue is immediately rinsed with water very few cell divisions occur in the cells adjacent to the wound. However if the wounded area is smeared with finely ground tissue of the same species considerable cell division occurs. This result led him to postulate the presence of substances which he called "wound hormones" in injured tissues which are required if cell division is to take place in the cells bordering a wound. Bonner and English (1938) have succeeded in extracting from dried bean pods and in partially purifying a compound which acts like a "wound hormone." They propose the name *traumatin* for this substance and have also devised a quantitative physiological method of testing for its presence.

Went (1938), on the basis of recent experiments, has postulated the existence of another group of hormones in plants for which he proposes the name *calines*. He considers that there are at least three such hormones: (1) *Rhizocaline*, produced in aerial organs, is necessary for root formation. (2) *Caulocaline* is formed in the roots but is necessary for elongation of stems or lateral buds. (3) *Phyllocaline*, apparently formed in the leaves, is necessary for leaf growth. Rhizocaline and caulocaline are believed to be effective only in the presence of auxins. This is a highly speculative, but nevertheless provocative hypothesis.

Similarly it has recently been postulated that flowering occurs only in the presence of a specific hormone which has been called *florigen* (Chap. XXXIII).

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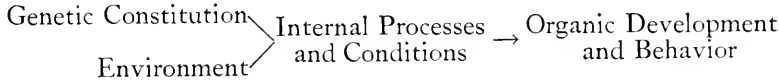
CHAPTER XXXIII

FACTORS AFFECTING GROWTH

Regardless of the habitat in which it is growing, whether a greenhouse, cultivated field, forest, prairie, mountain top, or lake bottom, a plant is continuously subjected to the variabilities of a complex, more or less interdependent set of environmental factors. The environment is the foster parent of every plant and animal and plays as indispensable a rôle in its development as do hereditary factors which have been transmitted to it from its biological parents.

The development and reactions of an organism are the result of the coordinated interplay of the hereditary factors and environmental conditions upon the internal physiological processes of that organism, as indicated in the following diagram:



Under the term "internal processes and conditions" are included all of the manifold variations possible in the physico-chemical conditions within cells and tissues, as well as the relative rates of the various fundamental physiological processes. With our present state of knowledge we are able to visualize only some of the grosser aspects of these processes with any great clarity. Most of the preceding chapters of this book have been devoted to a discussion of the internal processes and conditions in plants. The intermediate stages between genetic constitution and environmental factors on the one hand, and the development or reaction of the organism on the other, is a large and intricate one and is far from being bridged in terms of present day knowledge.

As a specific example of this principle we will recall the process of chlorophyll synthesis as it occurs in corn (maize). The usual varieties of this species contain the genetic factors which ordinarily induce chlorophyll formation. Certain environmental conditions, including light, are also necessary for its synthesis. A corn seedling developing in a dark room is devoid of chlorophyll, even if all the other environmental conditions necessary for chlorophyll formation are present. In a seedling growing in the light, how-

ever, interaction of the environmental factors with the hereditary mechanism will occur in such a way as to induce the process of chlorophyll synthesis in the leaf cells. As this example illustrates, while environmental conditions rarely have a direct influence upon the genetic makeup of an organism they often exert a profound influence upon the *expression* of its heredity.

So far, however, we have considered only one side of the story. Certain varieties of maize do not carry all of the genetic factors necessary for the development of chlorophyll. This trait is inherited in such strains of corn as a Mendelian recessive and hence is apparent only in plants homozygous for this factor. Even if all the environmental factors necessary for chlorophyll synthesis are present such seedlings cannot make chlorophyll and they develop as "albinos." As soon as the food stored in the seed is exhausted such albino seedlings die.

The genetic constitution of a given organism is a constant quantity and sets definite ultimate limits to the types of development and the reactions of which that organism is capable, beyond which no environmental condition can carry it. A potato plant, for example, remains unmistakably a potato plant in any environment in which it can survive.

The specific environment to which a plant is subjected also sets limits upon its development. For example under "short day" conditions, as discussed later in this chapter, a radish plant continues to grow vegetatively for an indefinite period of time. Although radish plants possess the hereditary capacity for reproductive development such an environment imposes a barrier to the expression of this particular potentiality. On the other hand, under long day-lengths, if other environmental conditions are favorable, a radish plant will flower and produce fruits within the course of a few weeks. Innumerable other examples of the environmental limitation of the expression of hereditary factors can be cited.

The full gamut of the hereditary potentialities of a species can never be realized until individuals of that species have been observed growing in each of a wide range of environmental complexes. Since most observations of the behavior of plants are made while they are growing under natural or cultural conditions which represent only a rather narrow range of variations in the environmental complex the many possible developmental reactions of a given species of plant are not always appreciated.

Environmental Factors Influencing Plant Growth.—The environment of living organisms is so complex as to defy any completely logical analysis. However, the important physical factors of the environment which ordinarily exert a more or less *direct* effect upon the growth and development of terrestrial plants can be recognized and are enumerated in the following list:

(1) Temperature (soil and air)
(2) Radiant energy
(3) Humidity
(4) Soil water
(5) Soil aeration
(6) Concentration of solutes in the soil solution
(7) Concentration of gases in the atmosphere
(8) Gravity
(9) Atmospheric pressure

The environment to which the roots are exposed is usually very different from that which the aerial organs of plants encounter. Because of reciprocal influences between the roots and tops of a plant, however, effects of any environmental factor upon the development or physiological processes of the roots almost invariably will be indirectly reflected in the behavior of the aerial organs, and *vice versa* (Chap. XXXIV).

Some important environmental factors such as precipitation (rain, snow, and hail), and wind are usually indirect in their influence on plants, operating through their effects on one or more of the direct factors listed above. Precipitation, for example, influences not only the soil water content, but also soil aeration and atmospheric humidity.

Many of the environmental conditions to which plants growing under out-of-door conditions are subjected are in turn influenced by more remote factors. The intensity and quality of impinging sunlight, for example, are functions of the angle of the earth's inclination to the sun (which varies with the latitude and season), the pitch of the slope upon which the light falls, and the direction towards which the slope faces. The soil water content is controlled not only by the precipitation, but by the surface run-off (which in turn is largely a function of the slope and the porosity of the soil), and by factors which influence the rate of evaporation such as air temperature, humidity, wind, and insolation. Similarly, with increase in altitude differences in such physical factors as the intensity, quality, and duration of radiant energy, soil and air temperature, atmospheric pressure, etc. are encountered.

Furthermore, complex inter-relationships exist among the medley of environmental factors which exert direct effects upon plants. Changes in the magnitude or duration of one factor seldom occur without inducing subsidiary changes in other factors. Increase in the intensity of radiant energy in any habitat results in an increase in soil and air temperatures; increase in soil water content diminishes soil aeration, etc.

Environmental factors influence plant development only because of their

effects upon internal physiological conditions which are also conditioned, as we have already seen, by the genetic makeup of the organism. Different combinations of factors often have a similar effect upon the internal physiological conditions in a plant. It is therefore entirely possible for approximately the same end result in terms of plant development to be induced by dissimilar combinations of environmental factors. The precept that the same internal physiological conditions, and the same developmental reactions can be brought about by different combinations of environmental factors is sometimes called the *principle of multiple causation.* This principle is well illustrated by the effects of various factors on the shoot-root ratio of plants (Chap. XXXIV).

In addition to the physical factors discussed above, plants are subject to the influence of another entirely different group of factors—the other living organisms in their environment. Among these are micro-organisms, animals, and other plants. Man himself, from the standpoint of a plant, is merely one of the factors in its environment. The influence of such *biotic factors* is not generally considered to come within the scope of a discussion of plant physiology, but their effects upon growth and development of plants is often as pronounced as the effects of physical factors. Biotic factors often operate as limiting factors in the survival or distribution of plants. The elimination of that once prominent tree species—the chestnut—from eastern North America by the chestnut blight disease is an example of the profound effects sometimes wrought by biotic factors.

In order to interpret the effect of changes in the magnitude of any one of the various factors influencing a process such as growth it is necessary to formulate certain guiding principles. In 1843 Liebig proposed his well known "law of the minimum," which was the first attempt at such a formulation. Liebig was thinking primarily of the effect of fertilizers upon the yield of crop plants when he suggested this "law," which states in essence that the yield is limited by the factor which is present in relative minimum. Blackman's "principle of limiting factors" as applied to photosynthesis (Chap. XXI) is essentially an elaboration of Liebig's principle.

Mitscherlich (1909) has proposed a somewhat different concept of the law of the minimum. He also was thinking principally of factors which influence the yield of crops. Like Liebig's principle, his statement affirms that factors present in minimal amounts are the most important in governing growth. His conception of the operation of the "limiting factor" may be stated as follows: "the increase in any crop produced by a unit increment of a deficient factor is proportional to the decrement of that factor from the maximum."

Both of these interpretations of the effect of minimal factors can be illustrated by a diagram (Fig. 131) in which it is supposed that five factors

are affecting growth, but that they are present in different relative intensities as compared with the maximum effectiveness.

According to Liebig's law of the minimum only an increase in factor *A* will cause an increase in the yield of a crop. According to Mitscherlich, increase in any one of these factors will cause an increase in yield. A unit increase in *A* will have the greatest effect, a unit increase in *B* the next greatest effect, etc. Factor *E* is so close to its maximum that a unit increase in it will have an almost negligible influence on yield. Mitscherlich's interpretation of the law of the minimum seems to be more nearly in accord with the results obtained in experiments on plants than Liebig's simpler formulation of this same principle.

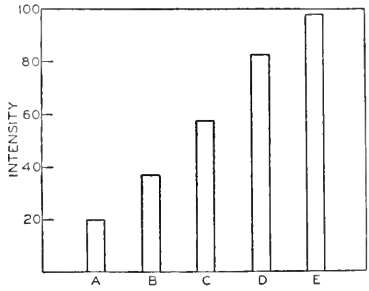


FIG. 131. Diagram to illustrate two interpretations of the "law of the minimum."

Effects of Temperature upon Growth.—The rate of every physiological process occurring in plants is markedly influenced by the all-pervading factor of temperature. Similarly the rate of growth, as measured in terms of any of the usual quantitative indices, is profoundly influenced by this factor. Temperature, however, exerts qualitative as well as quantitative effects upon the development of plants. In other words the structural development and physiological reactions of a plant may vary greatly, depending upon the temperature pattern of that plant's environment. Finally, whether or not a plant can survive in a given habitat often depends upon the temperature extremes which occur in that habitat.

1. *Effect of Temperature on the Rate of Growth.*—It has been customary to consider that there are three "cardinal" temperatures for growth, a *minimum*, an *optimum*, and a *maximum*, although these so-called cardinal points on the temperature scale may vary greatly with different species. While in a loose sense this is true, and such temperatures can be approximately determined by experimentation, they are by no means immutable. All three of these "critical" temperatures have been found to vary considerably with the stage in the development and the physiological condition of the plant, the time and rate of exposure, and other environmental conditions.

In general, the range of temperatures within which growth will take place varies considerably with the species. Arctic and alpine species may grow at the freezing point or even at temperatures slightly below, and their optimum growth temperature is often no higher than 10° C. Most species

of temperate zone origin do not grow appreciably at temperatures below 5° C. Their optimum growth temperature is usually about 25-30° C., and their maximum about 35-40° C. The cardinal growth temperatures of most tropical and sub-tropical species are still higher. For maize, a crop plant of sub-tropical origin, the minimum growth temperature is about 10° C., the optimum about 30-35° C., and the maximum about 45° C.

Leitch (1916) has made one of the few comprehensive studies of the effect of temperature upon the quantitative aspects of growth (see also Lehenbauer, 1914). She found that the rate of elongation of the roots of pea seedlings increased consistently with rise in temperature in the range of −2° to 29° C., and further that the rates within this range of temperatures, once established, showed little or no diminution with time. Above about 30° C., the higher the temperature, the lower the initial rate of growth, and the more rapidly the rate decreased with time. Elongation ceased entirely at temperatures of 45° C. or higher. In other words in the temperature range of 30-45° C., a distinct time factor effect was evident in the relation between temperature and growth.

The optimum temperature for the enlargement or elongation phase of growth is seldom the optimum for other phases of growth. Each stage in the development of a plant may and usually does have a different optimum temperature. In many species the optimum temperature for the germination of seeds is less than for vegetative growth, which in turn is often lower than the most suitable temperature for flowering and fruiting.

2. *Temperature Limitations upon Plant Survival.*—A clear distinction should be drawn between the extremes of temperature at which the growth of a plant ceases, and the extremes of temperature which that plant can endure without death resulting. The minimum temperature which a plant can tolerate without injury is almost invariably below that at which growth ceases; likewise plants can usually endure without lethal effects, at least temporarily, temperatures considerably in excess of the maximum at which growth occurs. A given plant, for example, may cease to grow when the temperature to which it is exposed rises to 40° C. Death, however, occurs only if the temperature of the plant is raised to some still higher temperatures, perhaps 55° or 60° C. Within this intervening range of temperatures the plant passes into a state of *heat rigor* in which it neither grows nor exhibits growth movements. Similarly there is a range of temperatures between the lowest temperature at which a plant will grow and its death point due to cold. Within this zone of temperatures the plant passes into a corresponding condition of *cold rigor.*

The upper and lower extremes of temperature which plants or plant organs

can endure vary greatly according to species and depend upon their capacity for heat resistance and cold resistance, respectively, as shown in the later discussion.

3. *Morphogenic Effects of Temperature.*—No two of the many and varied metabolic processes occurring in a plant are equally influenced by a change in temperature. Hence the morphogenic development of a plant is often markedly different under one set of temperature conditions than under another. Such effects upon the structural development of plants are the most complex and most striking of the many influences of temperature upon plants. Innumerable illustrations of the morphogenic effect of temperature could be mentioned, only one of which will be cited. Thompson and Knott (1933) have shown that if lettuce (white Boston variety) is grown at a temperature of 70-80° F. no heads form and the plants soon begin to develop flowerstalks. At the lower temperature range of 60-70° F., however, the plants form heads, and the development of flowerstalks is considerably delayed as compared with plants at the higher range of temperatures. A somewhat similar effect of temperature upon the development of celery is described in the last section of this chapter.

Cold Injury and Cold Resistance.—I. *Causes of Injury to Plants upon Exposure to Low Temperature.*—Several types of injury may occur in plants as a result of exposure to conditions which often prevail during cold seasons:

(1) Suffocation.—When covered for long periods during the winter with densely packed or encrusted snow some low-growing species of plants may suffer from a deficient oxygen supply. This is reported to sometimes result in serious injury to wheat in parts of Russia, but in general is not a phenomenon of very frequent occurrence.

(2) Desiccation.—Relatively high winter transpiration rates in evergreens during a period when absorption of water can proceed only at a relatively slow rate often lead to a type of injury frequently called *winter-killing* (Chap. XIV). Injury under such circumstances is due to desiccation of the tissues. A similar type of injury may result to some plants, especially herbaceous species, as a result of frost heaving of the soil. Such heaving often tears the roots loose from the soil or, in extreme cases, may even result in breaking them. If environmental conditions favoring high transpiration rates intervene before the root system can be securely re-established in the soil the plant may be so severely desiccated that death or marked injury results. This is often a serious source of injury to winter wheat during "open" winters. One of the advantages of mulching plants with straw, leaves, etc. during the winter months is that it greatly reduces frost heaving of the soil.

(3) Chilling Injury.—Many species of plants, particularly those which

are native to tropical or sub-tropical regions, are killed or seriously injured by relatively low temperatures *above* their freezing point. This type of low temperature injury is often called *chilling injury*. For example, Sellschop and Salmon (1928) found that exposure to a temperature of 0.5 to 5.0° C. for 24 to 36 hours was fatal or markedly injurious to rice, velvet beans, cotton, peanuts, and Sudan grass. Species which were only slightly injured included maize, sorghums, watermelons, and pumpkins, while soy beans, buckwheat, tomatoes, and flax showed no evidence of injury from such a chilling. The cause of such pronounced effects of low but not freezing temperatures undoubtedly lies in disturbances which are induced in the metabolic activities and physiological conditions within the cells, but little or no precise information is available regarding the exact nature of such disturbances. In general the longer the exposure of such plants to chilling temperatures, the greater the resulting injury.

(4) Freezing Injury.—Many plant tissues are killed or irreparably injured when they are exposed to temperatures which are low enough to cause ice formation to take place within them. This is the most frequent and fundamental type of low temperature injury in temperate climates and the remainder of the discussion will be devoted to it. Many plant organs, however, can endure subjection to such conditions. Plants or plant organs of which this is true are said to be *cold resistant, frost resistant*, or *hardy*.

II. *Mechanism of Ice Formation in Plant Tissues.*—When water is gradually cooled, freezing usually does not begin at 0° C. but only after its temperature has dropped from a fraction to several degrees below its freezing point. In other words the water is first *undercooled* before freezing begins. With the initiation of ice crystal formation heat of crystallization is released, resulting in a rise of the temperature of the water to its freezing point. Similarly the water in plant tissues usually does not freeze until after the tissues have been undercooled. Some plant tissues can be undercooled as much as 15° C. below their true freezing point before crystallization of water begins, but for most plant tissues the undercooling is only a few degrees. The actual freezing points of plant tissues, however, are seldom below −5° C.

Because of their capacity for undercooling some plants normally susceptible to frost injury can survive short exposures to freezing temperatures without injury. This is true, for example, of certain species of cacti, which often can be undercooled 10-15° C. without any formation of ice crystals. Once such tissues actually freeze, however, they are killed or seriously injured.

When plant tissues freeze, ice formation often takes place in the intercellular spaces. The crystals enlarge at the expense of water which diffuses from the abutting cells. There are a number of conditions, however, under which

the crystallization of water takes place within the cells, either in the vacuole, or the cytoplasm, or both. The factors which determine whether freezing will occur within the cells or between them are not well understood. Freezing in the intercellular spaces is apparently much less often injurious to cells than freezing within the cells.

III. *Causes of Freezing Injury in Plants.*—A number of suggestions have been made regarding the possible causes of freezing injury to plant cells, the principal ones being the following:

(1) Ice formation in the intercellular spaces results in withdrawal of water from the cells. The consequent dehydration results in disorganization and death of the protoplasm (Molisch, 1897).

(2) The ice formed in the intercellular spaces results in mechanical compression of the cells which in turn causes deformation and death of the protoplasm (Maximov, 1914).

(3) Withdrawal of water from the cells due to the formation of ice crystals in the intercellular spaces results in an increase in the concentration of electrolytes in the cell sap which may have a "salting out" or other destructive effect on the protoplasmic proteins (Harvey, 1918, and others).

(4) Ice crystals may form within the cell, resulting in compression or laceration of the protoplasm or other destructive effects (Stiles, 1930, and others).

(5) Death occurs, not at the time of ice formation, but as a result of the subsequent thawing of the tissue. This idea, originally sponsored by Sachs in 1860, largely dropped into disrepute but has been revived by the findings of Iljin (1933) and others that some plant tissues which can withstand freezing are killed by rapid thawing. Death is apparently due to various types of mechanical disturbances attendant upon the entry of water into the cells upon thawing.

Most modern workers seem to favor the concept that injury to protoplasm during freezing is fundamentally due to mechanical effects of ice formation either within or between the cells rather than to dehydration *per se* or to chemical effects. The ultimate effect of such mechanical disturbances is presumably the disruption of the delicate organization of the protoplasm.

IV. *Hardening.*—Exposure of many species to low temperatures just above the freezing point results in a marked increase in their cold resistance. This phenomenon is called *hardening.* Crop plants such as cabbage which are to be planted early in the spring are often hardened artificially before being set out in the field. This is usually done by transferring the cabbage seedlings from the greenhouse to a cold-frame for a few days before they are transplanted into the field.

According to Harvey (1930) and others the threshold temperature for inducing hardiness is about 6° C., at least in some species. He found that exposure of cabbage plants to a temperature of 0° C. for one to four hours per day kept the plants in a hardy condition even if they were exposed to temperatures of from 10° to 20° during the remainder of the day. This indicates that as long as outdoor temperatures fall to about 0° C. for a few hours each day, species with the capacity for cold resistance probably remain resistant to injury at considerably lower temperatures.

Seasonal variations in hardiness are of normal occurrence in the organs of temperate zone species which are exposed to freezing temperatures during winter months. The leaves of evergreen plants are not cold resistant during the summer, but pass into the hardened condition in the autumn, probably remaining continuously in this state during the winter. In the spring they pass through a dehardening process and lose their cold resistance. The living cells of the exposed stems of deciduous species undoubtedly pass through a similar seasonal cycle of hardening and dehardening.

Among the physiological conditions which seem to be often associated with the property of cold resistance in plant tissues are (1) relatively low water content of the tissues, (2) accumulation of soluble carbohydrates in the cells accompanied by an increase in their osmotic pressure, and (3) an increased proportion of unfreezable ("bound") water in the tissues.

Heat Injury and Heat Resistance.—I. *Causes of Injury to Plants at Relatively High Temperatures.*—Several types of injury result to plant cells either directly or indirectly from relatively high temperatures:

(1) Desiccation Injury.—High leaf temperatures resulting either from intense insolation or high air temperatures, or both, may result in excessive rates of transpiration. A relatively high rate of water loss, particularly at times when the rate of absorption of water is sluggish, often leads to death of some or all of the leaves or branches on a plant as a result of desiccation. In extreme cases entire plants are killed in this way.

(2) Injury Resulting from Metabolic Disturbances.—Relatively high temperatures often induce various types of metabolic disturbances which are detrimental or even fatal to plants. One important example of such an effect has already been described (Chap. XXIX). With rise in temperature increase in the rate of photosynthesis usually fails to keep pace with increase in the rate of respiration. Relatively high temperatures therefore frequently cause a stunting of plants because a disproportionate amount of the foods manufactured is consumed in respiration. Maintenance of such a condition for extended periods often results in the death of plants.

(3) Direct Thermal Effects upon the Protoplasm.—The thermal death

point of most living active plant cells is in the approximate range of 50-60° C. The exact temperature at which death of the protoplasm will occur depends upon the length of the period during which the cells are warming up to the lethal temperature. For example, according to Lepeschkin (1912), if the leaf epidermal cells of *Rhoeo discolor* were heated at such a rate that death occurred in four minutes the lethal temperature was 72.1° C. If the rate of warming was so slow that death did not take place until 150 minutes had elapsed the thermal death point was only 52.0° C.

Air temperatures in temperate regions seldom exceed 40° C., and although the temperature of an insolated plant organ often exceeds that of the atmosphere, lethal temperatures are seldom attained for reasons discussed in Chap. XII. The surface temperatures of some soils, however, may attain values of 70° C. or even higher when exposed to intense insolation. Attempts to reforest denuded areas in certain regions have sometimes failed because of such high soil surface temperatures. The living cells of the stems of the young trees which had been set out were killed at the soil line by contact with soil at a temperature above their thermal death point, thus causing death of the entire transplant. On the other hand, many woody species are habitants of semi-desert regions in which high soil temperatures often prevail. The stems of such species are obviously more heat resistant than those which are injured or killed by contact with hot soils.

Similarly lichens, certain species of mosses, and other species which grow on rock cliffs exposed to the full glare of the sunlight often possess considerable heat resistance because of the heating effect of the sun's rays upon their rock substratum.

Another example of direct heat injury to plants is often evident after a "ground" forest fire sweeps through a woods. Such fires burn only fallen leaves and branches on the forest floor and are often without any apparent immediate effects upon living trees and saplings. Subsequently the tops of many of the trees on an area burned over by such a fire often die as a result of the killing of an encircling zone of living cells at the base of the trunk by the high temperatures to which they have been exposed.

II. *Theories of Heat Injury.*—The most generally advocated theory of the cause of direct heat injury to plant cells is that it is due to a coagulation of the protoplasm. Many proteins are heat coagulable and since protoplasm is largely composed of proteins it seems highly probable that heat injury to cells is due largely if not entirely to the coagulation of protein components of the protoplasm (Lepeschkin, 1935).

Several other theories of the mechanism of heat injury to plant cells have been advanced. It has been suggested, for example, that heat injury is due

to a destruction of enzymes or to changes in the physical state of the lipids. The preponderance of the evidence at present available, however, seems to favor the coagulation of protoplasm theory as an explanation of the mechanism of heat injury to protoplasm.

III. *Heat Resistance.*—Certain types of tissues are more resistant to heat injury than others. Tissues low in water content generally can endure relatively high temperatures better than those of which the contrary is true. Dry seeds and spores of some species have endured exposure to temperatures of 125° C. and even higher without loss of germinative capacity.

In some plants otherwise susceptible tissues are protected against heat injury because they are enclosed within tissues which have a low thermal conductivity. In a forest which has been swept by a ground fire, to continue an earlier example, it is usually noticeable that some of the trees have escaped injury while others have been killed. Of individuals of the same species older trees are more likely to survive than the younger ones. In mixed stands pines are more likely to escape injury than hardwoods. These differences in susceptibility to injury from ground fires are apparently correlated with the thickness of the bark layer (largely cork) which acts as an insulation between the inner living tissues and high external temperatures. The bark is thicker on old trees than on younger trees of the same species. Similarly the bark of pines is usually thicker than that of most hardwoods.

Effect of Radiant Energy on Growth.—The spectrum of radiant energy (Fig. 79) ranges from the very long electric waves to the infinitesimally short cosmic waves. All kinds of radiant energy, including light, vary in several different ways, the most important of which are: (1) intensity, (2) quality and (3) duration (Chap. XIX).

Light is absolutely essential to all green plants because of its primary rôle in photosynthesis. Numerous other effects of light upon physiological conditions and processes in plants have been discussed in previous chapters. Among these are: (1) chlorophyll synthesis, (2) stomatal action, (3) anthocyanin formation, (4) temperature of aerial organs, (5) absorption of ions, (6) permeability, (7) rate of transpiration, and (8) protoplasmic streaming. Several of the most important effects of light upon plants remain to be discussed in this and the following chapters.

In the present chapter we will deal with a few of the more striking examples of effects of differences in the intensity, quality, and duration of light and other forms of radiant energy upon the morphogenic development of plants.

1. *Intensity.*—Variations in the intensity of sunlight or artificial sources of mixed radiations are almost invariably accompanied by at least minor varia-

tions in the *quality* of light. Even under experimental conditions it is difficult to provide differences in light intensity which are qualitatively identical, and this fact must be considered in evaluating the results of all such experiments.

We shall first consider the development of plants as it occurs in the complete absence of light but under such conditions that food is available to the growing parts from a storage organ such as a seed, tuber, or bulb (MacDougal, 1903). Seedlings of dicots which have developed in total darkness

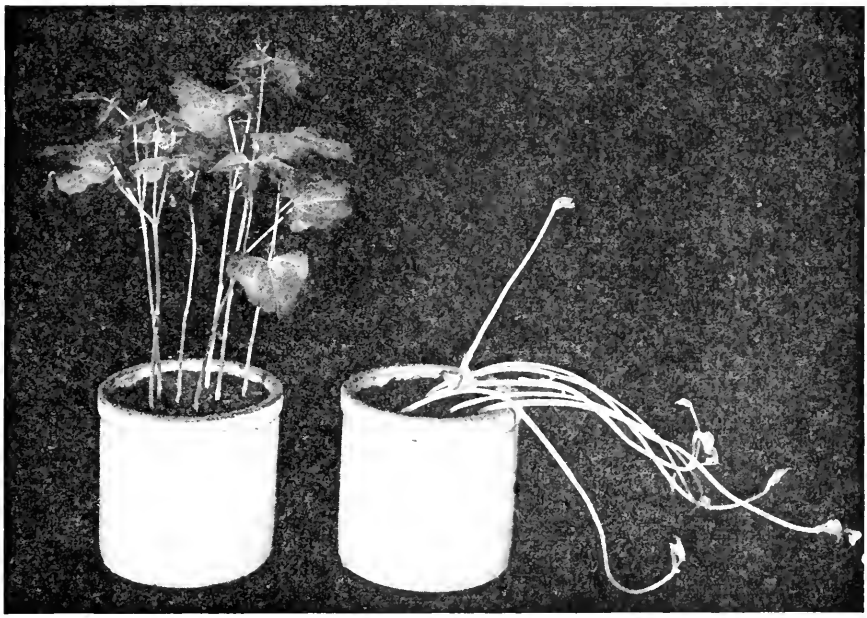


FIG. 132. Seedlings of bean (*Phascolus vulgaris*) about two weeks old grown in light (left) and in total darkness (right).

have spindling, yellowish, succulent stems on which the leaves fail to expand (Fig. 132). Their root systems are also relatively poorly developed. Individuals of the same species developed in full daylight, on the contrary, are of normal stature, bear fully expanded green leaves, have relatively larger root systems, and develop a less succulent type of stem structure.

When seedlings of monocot species develop in total darkness the chlorophyll-free leaves are relatively narrow and more attenuated than the leaves of similar plants which have developed in the light.

The distinctive morphogenic development of plants which have grown in total absence of light is called *etiolation*. Exposure of plants to light of a

very low intensity is sufficient to prevent the development of any pronounced earmarks of etiolation. When seedlings grow in very weak light the leaves expand and synthesize chlorophyll and the internodes do not elongate as much as on similar plants growing in the dark, although the plant will usually show a more attenuated development than in stronger light. Even a short *temporary* exposure to light often results in the development of a much more nearly normal configuration in plants which are subsequently returned to a dark room (Priestley, 1925, 1926). This suggests that substances of the general nature of hormones are synthesized in plants under the influence of light, and that it is the absence of such substances which permits the development of the characteristic symptoms of etiolation. This supposition is supported by the fact that the elongation rate of seedlings, including those parts which remain underground, is immediately checked upon emergence of the hypocotyl or plumule into the light.

The attenuated growth of the leaves (monocots) and stems (dicots) of etiolated plants appear to be due chiefly to an increase in the length of their component cells as compared with cells of similar tissues developed in the light. Some of the increased length of most etiolated plant organs is also due, however, to more frequent cell divisions in organs growing in the absence of light than in those which are illuminated.

The phenomenon of etiolation indicates convincingly that light exerts a retarding effect upon the enlargement phase and probably also on the cell division phase of growth. Maturation, on the other hand, appears to be favored by exposure to light.

The retarding effect of relatively high light intensities upon growth, however, is usually at least in part a temperature and desiccation effect. High light intensities favor rapid transpiration rates which in turn almost invariably result in a reduction in the water content of the plant. Reduced hydration of plant cells, if at all marked, always causes a retardation of cessation of the division and enlargement phases of growth.

For a more comprehensive picture of the effects of different light intensities upon growth we must turn to investigations such as that of Popp (1926) on soy beans. He grew plants of this species under six different light intensities averaging 4285, 1536, 560, 390, 250, and 26 foot candles respectively. While during the initial period of growth the rate of stem elongation varied inversely with the light intensity, when growth was considered for a 7 weeks period a somewhat different relation was found to hold. For such periods the greatest height was attained at intermediate light intensities, in this particular experiment at 560 foot candles. The thickness of the stems was found to vary

directly with the intensity of the light, while the best general development of leaves, flowers, and fruits occurred in the greatest light intensity used, which was approximately half the intensity of the noon sun on a clear summer's day (Chap. XIX).

Somewhat similar results were obtained by Shirley (1929, 1936) who studied the effects of differences in light intensity upon the development of a number of species. In general the absolute dry weight, percentage of dry matter in the tops, rigidity of the stem, and leaf thickness all increased with increase in the intensity of light up to full sunlight, providing other factors were not limiting growth. Maximum height of the plants and maximum leaf area were attained, on the other hand, at light intensities considerably below that of full summer sunlight. Low light intensities also resulted in a considerable delay in the time of maximum flowering and fruiting.

In general the results of these and other similar investigations indicate that maximum height and leaf area are attained at light intensities which are considerably less than full summer sunlight. Relatively high light intensities result in most species in shorter internodes, plants of lower stature, and smaller leaves, but the dry weight, number and size of the branches, size of the root system, and production of flowers and fruits is greater than in weaker light intensities. Many species show increased growth in terms of dry weight increment with increased light intensity up to 100 per cent of summer sunlight, if no other factor is limiting. All phases of the growth of typical shade species are usually retarded, however, by high light intensities.

2. *Quality.*—Because of the experimental difficulties involved no entirely critical study of the exact effects of different light qualities upon the development of the higher plants has ever been carried out. Various qualities of light for experimental work on plants are usually obtained by allowing sunlight or artificial light to pass through filters of colored glass, gelatin, or other materials. If the energy spectrum of the light source and the transmissive properties of the filter are known the quality of light falling upon the plants placed under such filters can be determined. It is difficult, however, to select or adjust filters so that the different qualities of light used are all equal in intensity. Furthermore most qualitatively different light sources which can be experimentally produced either by means of filters or in other ways are of relatively low intensity, which necessarily limits the comprehensiveness of studies which can be made of this problem. For these reasons most conclusions regarding the influence of different light qualities upon growth in plants are of restricted application.

All experiments upon the effect of limited ranges of wave lengths of light

lead to the not surprising general conclusion that the full spectrum of sunlight is more satisfactory for the development of plants than any spectral portion thereof, or than any known artificial source of illumination.

As a rule plants grown under the longer wave lengths of the visible spectrum resemble etiolated plants more or less closely. Internodes and petioles elongate more than in white or blue-violet light, and the leaves fail to expand fully (Popp and Brown, 1936 a).

Plants allowed to develop in a blue-violet light are usually similar in gross morphology to those allowed to develop in "white light," although they are often smaller and more compact. Petioles and internodes are shorter than when plants develop in red light, and leaves expand in an approximately normal fashion. Apparently the development of their usual configuration by plants is largely due to an effect of the shorter wave lengths of the visible spectrum, since when these wave lengths are missing from the incident light disproportionate development of the organs occurs as previously described.

Recent experiments of Johnston (1932), however, show that tomato plants exposed to a relatively high intensity of infrared radiation have longer internodes, larger leaves, and less chlorophyll than similar plants receiving the same quantity of visible radiation, but no infrared. The influence of infrared radiation upon plants results at least in part and perhaps entirely from its heating effects.

All wave lengths of ultraviolet shorter than those found in the sunlight which impinges upon the earth's surface have a retarding effect on growth and often a destructive influence on plants (Popp and Brown, 1936 b). The effects of the sunlight ultraviolet which naturally falls upon plants are, in general, very similar to those of the blue-violet region of the visible spectrum.

The majority of numerous investigations indicate that X-rays exert only destructive effects upon plants, although a few workers are of the opinion that very weak doses sometimes have a stimulatory effect (Johnson, 1936).

The exact influence of different light qualities can only be completely evaluated by testing the effects upon plants of exposure to relatively narrow bands of light of equal intensity in various parts of the spectrum. No critical experiment of this sort has ever been performed upon any of the higher plants. Meier (1936), however, exposed cultures of an unicellular alga (*Stichococcus bacillaris*) to full daylight, to infrared radiation, and to five narrow ranges of wave lengths under artificial light. The radiation provided was equal in intensity for all cultures except those exposed to daylight. Growth was measured in terms of cell multiplication during a two weeks exposure. Increase in the number of cells in the daylight cultures was more than fourfold, in blue light (400-520 $m\mu$) over threefold, and in both red (600-750 $m\mu$)

and yellow light (550-620 $m\mu$) more than twofold. In cultures exposed only to infrared (850-1200 $m\mu$) or kept in complete darkness little change occurred in the number of cells present, but in green light (500-560 $m\mu$) a diminution occurred in the number of cells in the culture. The green region appeared therefore to have an actual destructive effect upon algal cells, and perhaps may exert such an effect upon plant cells generally.

3. *Duration of the Light Period.*—In all parts of the world except the tropics and sub-tropics marked seasonal variations occur in the length of the daylight period. At 39° N. latitude (approximately that of Washington, D. C.) for example, on the shortest day (December 21) the sun shines for only about 9½ hours; on the longest (June 21) for about 15 hours. The actual daylight period is always somewhat longer than the number of hours of possible sunshine. At higher latitudes the annual variation in day-length is greater, at lower latitudes less (Table 57). Only in recent years, however, has any systematic attempt been made to evaluate the influence of this factor upon plant development. Investigations during the last two decades have shown that the phenomenon of *photoperiodism,* as the development of plants in relation to the length of the daily light period is now called, is one of the most notable of all reactions of plants to their environment.

TABLE 57—APPROXIMATE NUMBER OF HOURS OF SUNSHINE POSSIBLE AT VARIOUS DEGREES OF NORTH LATITUDE

Degrees N. latitude	Approximate latitude of	Hours of sunshine possible			
		Dec. 21	Mar. 21	June 21	Sept. 21
25	Key West, Fla.	10.6	12.2	13.7	12.2
27	Palm Beach, Fla.	10.4	12.2	13.9	12.2
29	San Antonio, Tex.	10.3	12.2	14.0	12.2
31	Mobile, Ala.	10.1	12.2	14.1	12.2
33	Charleston, S. C.	10.0	12.2	14.3	12.2
35	Memphis, Tenn.	9.8	12.2	14.5	12.2
37	San Francisco, Cal.	9.6	12.2	14.7	12.2
39	Washington, D. C.	9.4	12.2	14.9	12.2
41	Omaha, Neb.	9.2	12.2	15.1	12.2
43	Milwaukee, Wis.	9.0	12.2	15.4	12.2
45	Portland, Ore.	8.8	12.2	15.6	12.2
47	Duluth, Minn.	8.5	12.2	15.9	12.3
49	Vancouver, B. C.	8.2	12.2	16.2	12.3

The foundations of our present knowledge of photoperiodism were laid when Garner and Allard (1920) observed the behavior of plants of the "Maryland Mammoth" variety of tobacco while growing in a greenhouse

during the winter months. This commercially valuable variety of tobacco does not ordinarily blossom in the summer while growing out-of-doors in the latitude of Washington, D. C. When sown in a greenhouse during the winter months, although the plants which develop were much smaller (field grown plants in the summer attained heights of ten to fifteen feet; greenhouse plants grown in the winter did not exceed five feet in height) they blossomed profusely and produced excellent crops of seed. These observations led to the hypothesis that the dissimilar development of the tobacco plants during the two seasons was due to the difference in the length of day, relatively

FIG. 133. Effect of the length of the photoperiod on flowering of lettuce, a long day species. Photograph from Arthur *et al.* (1930).

short days apparently favoring reproductiveness in this species. Subsequent more critically performed experiments confirmed this hypothesis.

Later these experiments were extended to include a large number of additional species. "Short day" conditions were provided, during the summer months, by transferring the plants to a dark house after exposure to the desired number of hours of daylight, while exposure to the normal summer day-length provided "long day" conditions. Long day-lengths were obtained during the winter months by supplementing the hours of daylight with the necessary number of hours of artificial illumination. The exact intensity of the artificial light used does not appear to be critical; intensities no greater than one-thou-

sandth of that of sunlight were found to be adequate for the production of photoperiodic effects on growth. More recently intensities as low as 0.1 foot candle have been found to induce photoperiodic effects, at least in some species (Withrow and Benedict, 1936).

The fundamental principles of photoperiodism as discovered by Garner and Allard have been confirmed by a number of later investigators. The most important effect of day-length is upon the reproductiveness of plants. Although most plants exhibit better vegetative development when exposed to long photoperiods than short ones this statement does not hold for reproductive development. In general plants fall into three groups:

(1) "Long-day" species. Species in this group flower more or less readily in a range of day-lengths *longer* than a certain *critical photoperiod.* Many

FIG. 134. Effect of length of the photoperiod on flowering of salvia, a short day species. Photograph from Arthur *et al.* (1930).

such species flower and fruit even in continuous illumination. At shorter day-lengths than the critical these plants produce solely vegetative organs, in many species developing only rosettes (Fig. 133).

(2) "Short-day" species. Species in this group flower more or less readily in a range of day-lengths *shorter* than a certain critical photoperiod. Under longer photoperiods, however, they develop only vegetatively (Fig. 134).

(3) "Indeterminate" species. The species falling into this category exhibit no critical photoperiod, most of them developing both vegetatively and reproductively over a wide range of day-lengths (Fig. 135).

The classification of plants into long-day and short-day types depends, in brief, upon whether the critical period represents the lower or upper limit of day-length conducive to reproductive growth. The critical photoperiod

is not the same for all species but for most lies within a range of 12-14 hours. In this range of day-lengths many species of both the long-day and short-day types will flower, although maximum reproductiveness is not usually attained in photoperiods of this duration. According to Garner (1933) a day-length of 14 hours comes closest to a dividing line between plants of these two groups.

In order to determine in which of the three groups a species belongs it usually is sufficient to expose some individuals of that species to a relatively short day-length of about 10 hours, and others to a decidedly long one of

FIG. 135. Effect of length of the photoperiod on flowering of buckwheat, an indeterminate species. Photograph from Arthur, *et al.* (1930).

about 18 hours. If flowering fails to occur or is markedly retarded under the short day-length but occurs under the long day-length the plant belongs to the long-day group. If the contrary is true the plant is a short-day species. If blossoming occurs under both day-lengths the plant can be assigned to the indeterminate group.

In many species a difference of one hour in the length of the daily photoperiod, or sometimes even less, determines whether the plant will blossom or will be restricted to the vegetative phase of development. Garden balsam

(*Impatiens*), for example, flowers readily when exposed to a day-length of 14 hours, is greatly delayed in flowering by a day-length of 13½ hours, and at day-lengths of shorter duration is strictly vegetative. While the dividing line between the range of photoperiods which favors reproductive development and that which does not is not as sharp in all species, usually a fairly definite critical day-length can be recognized.

In addition to the Maryland Mammoth variety of tobacco, other examples of short-day plants are cosmos, salvia, coleus, asters, dahlia, poinsettia, chrysanthemum, nasturtium, violets, and all early spring or late summer blooming wild flowers of the temperate zone.

Examples of long-day plants include spinach, beets, radish, lettuce, grains, timothy, clover, hibiscus and all late spring and early summer blooming wild flowers of the temperate zone.

Some of the better known examples of "indeterminate" species are sunflower, dandelion, chickweed, tomato, cotton, and buckwheat.

The season of the year at which a plant will bloom is largely controlled, at least in temperate regions, by its type of reaction to day-length conditions. The natural blooming period of long-day plants is in the late spring and early summer. Short day species which can grow at relatively low temperatures bloom in the early spring, the flower buds in many such species being formed during the preceding autumn. The majority of the members of this group do not develop flowers until the advent of the shortening days of late summer or early autumn. This latter type of behavior is invariably found in the annual species belonging to this group and is characteristic of many perennial species as well. Indeterminate species, on the other hand, may flower at almost any season during which other environmental conditions are favorable.

The ecological distribution of plants is also limited in part by photoperiodism. Tropical and sub-tropical species are mostly short-day plants. Species growing at high latitudes (60° and farther north) are mostly of the long-day type. In temperate zones both long-day and short-day species flourish, but flower at different seasons as already described. Indeterminate species can reproduce over such a wide range of day-lengths that their distribution is limited by other factors than the daily duration of the light period.

Garner and Allard (1923) also succeeded in demonstrating that the reaction of plants to differences in day-length may be highly localized. They arranged two similar branches of a cosmos plant in such a way that one was exposed to a short day-length, and the other to a long-day length. The former of these two branches soon produced flowers and fruits, and subsequently died (cosmos is a short-day plant), but the latter continued to grow vegetatively

for an indefinite period. Similar localized reactions of plants to the length of the light period can be demonstrated in other species.

The influence of the duration of the daily photoperiod upon reproductiveness is by far the most outstanding, but by no means the only effect of this climatic factor on growth. Some of its other more important effects are upon: (1) production of storage organs (Zimmerman and Hitchcock, 1929), (2) sexual expression (Schaffner, 1923, 1930), and (3) rejuvenation (Garner and Allard, 1923).

Species native to arctic regions, as well as a number of crop species (tomatoes, grains, berries) develop well under summer conditions of continuous or nearly continuous daylight. In general development of such plants at high latitudes takes place more rapidly than in regions of shorter summer day-lengths. Harvey (1922) and others have shown that a number of annual species such as wheat, oats, flax, cotton, buckwheat, white clover, peas, and beans can complete their life cycle from seed to seed under continuous artificial illumination. Presumably only species of the long-day or indeterminate types will show such a reaction to continuous illumination and even some of them are injured or killed when exposed to such conditions (Arthur, 1936).

The results of the numerous experiments which have been performed on photoperiodism indicate clearly that length of day influences not only the quantity of photosynthate produced, but the use which the plant makes of the compounds which it synthesizes. Present indications are that photoperiodic effects in plants depend at least in part, and perhaps entirely, upon a hormonal mechanism. The Russian investigator Cajlachjan (see Garner, 1937) has shown that metabolic processes caused by changes in length of day which lead to flowering occur in the leaf tissues, but are entirely distinct from carbohydrate synthesis. The influence of these processes is apparently transferred from the leaves to growing points by material substances of a hormonal nature. The name *florigen* has been proposed by Cajlachjan for the postulated "flower hormone." Results of Loehwing (1938 a) also point in this direction. When the tops of soy beans of the short-day Ito San variety were exposed to 9 hours daily illumination, and the base to 14 hours, flowers formed only on the tops. When the base received a daily photoperiod of 9 hours and the tops of 14 hours, flowers developed only on the bases. However if the top was first defoliated and exposed to a 14 hour day-length, flowers developed on it if the base was exposed to a 9 hour day-length and kept exflorated. Apparently under a short photoperiod specific substances which are necessary for flowering were synthesized in the leaves, and, under the conditions of this experiment, these hormone-like substances were translocated into the tops of the plant. Similarly if the base was defoliated and exposed to

14 hours of illumination per day, while the top received 9 hours and was kept exflorated, flowers developed only on the base.

The results of a recent investigation by Zimmerman and Hitchcock (1936) also point strongly toward a hormonal mechanism for at least some types of photoperiodic effects. When Jerusalem artichokes developed under long-day and short-day conditions during the summer months the former produced underground stems but no tubers, the latter tubers but no underground stems. If, however, the stem tips only were subjected to short-day conditions by covering them with black cloth caps, while the rest of the plant received the normal summer day-length, they behaved as if the entire plant was subjected to short-day conditions, *i.e.* produced tubers. Obviously differences in response of the stem tips to different photoperiods are in some way communicated to the underground organs of the plant and there exert a regulatory influence upon their development. Such an effect can be most easily visualized in terms of a hormonal mechanism.

Important practical applications of the principles of photoperiodism have been made, especially to the growing of floricultural greenhouse crops. Short-day species such as chrysanthemums can be brought into bloom earlier in the fall by decreasing the length of their daily exposure to light. Most floricultural species, however, are of the long-day type. The time required for the majority of such species to attain the flowering stage can be shortened during the winter months, often very markedly, by increasing the day-length with supplementary artificial illumination. This procedure has been found to be commercially practical with a number of species (Laurie and Poesch, 1932).

The Water Factor in Growth.—The dynamic condition of the water in a plant is largely controlled by the opposing effects of the processes of transpiration and water absorption as already described in Chap. XVIII. Whenever the rate of the former process exceeds the latter for any appreciable period of time the volume of water within the plant shrinks. This results in a diminution in cell turgidity, an increase in the diffusion pressure deficit of the water in the cells, and a decrease in the hydration of the protoplasm and cell walls. A decrease in the hydration of the protoplasm in the cells of any meristematic tissue invariably results in a cessation or checking of one or more of the phases of growth.

Contrariwise, a shift in environmental conditions which brings about, directly or indirectly, an increase in the hydration of the protoplasm of a meristem usually results in an increased rate of growth if no other factors are limiting.

The water factor as it affects growth processes is primarily an internal

in Chap. XXV and this topic will not be considered further. Similarly the part played by nitrogen in the growth of plants has already been discussed in Chap. XXVI.

The Influence of Atmospheric Gases on Growth.—The biologically important gases which are invariably present in the atmosphere are oxygen, carbon dioxide, and water-vapor. The first two of these are so nearly constant in their atmospheric concentrations that they seldom need to be considered as variables in interpreting the developmental behavior of plants under natural conditions. The only exception to this statement is the occasional enrichment of the lower strata of the atmosphere with carbon dioxide as a result of the respiration of soil micro-organisms (Chap. XXI). The water-vapor content of the air, on the other hand, varies considerably. The pronounced influences of this factor upon transpiration, the internal water relations of plants, and indirectly upon growth have already been considered.

Sometimes gases other than those normally present in the atmosphere become a part of the environment of plants. Smelters, for example, release considerable quantities of sulfur dioxide into the atmosphere. This gas, in any appreciable concentration, is highly toxic to plants, hence the countryside in the vicinity of smelters is often virtually denuded of vegetation. Most species of plants are injured by an exposure of only one hour to an atmosphere containing as little as one part of this gas in a million (Zimmerman and Crocker, 1934).

Escaping illuminating gas often produces injurious or lethal effects upon plants. Leaky gas mains sometimes cause the death or injury of shade trees along city streets. Similarly injury or death of greenhouse plants has sometimes been found to result from leakage of gas from underground mains or other sources.

Manufactured illuminating gas contains both carbon monoxide and ethylene, the former being present in much larger proportions than the second. Ethylene, however, is the chief toxic constituent of artificial illuminating gas. Prolonged exposure to even very small concentrations of this gas in the atmosphere results in death or profound physiological disturbances in most species of plants.

Some species, of which the tomato is an example, are exceedingly sensitive to ethylene. The leaves of tomato plants soon show *epinasty* (Chap. XXXVII) at concentrations as low as 0.1 part of ethylene to a million of air. A few species are even more sensitive to ethylene (Crocker, *et al.*, 1932).

Since such minute concentrations of ethylene are far less than can be detected by odor, or even by chemical tests, the simplest method of detecting this gas when present only in traces is to stand several young potted tomato

plants in the greenhouse or room to be tested for several days. If traces of ethylene are present epinasty of the leaves will soon become apparent (Fig. 136).

Physiological Preconditioning.—The growth performance of a plant at any stage of its development is not only continuously influenced by its hereditary makeup and by the prevailing environmental conditions, but often shows a lingering effect of the environmental conditions to which it has been exposed

FIG. 136. Epinasty in tomato due to exposure to ethylene. Plant on right exposed to 0.1 parts per million of ethylene for 48 hours; plant on left kept under same conditions in an ethylene-free atmosphere. Photograph from Crocker, *et al.* (1932).

during some previous stage in its life history. The induction within a plant of certain internal conditions which carry over into a later stage of its life cycle is often designated by the term of *physiological preconditioning.* Undoubtedly such effects are often due to certain compounds which are synthesized during an earlier stage in the development of a plant. In some physiological preconditioning phenomena these compounds may be foods, in others they are probably compounds of the general nature of hormones. Our dis-

cussion will be limited to two of the more striking examples of such phenomena.

For some years it has been known that exposure of the soaked seeds of winter wheat to temperatures just above the freezing point for a considerable period speeds up the reproductive development of the resulting plants. For example, if soaked seeds of the Turkey Red variety of winter wheat were exposed to a temperature of 1° to 30° C. for 9 to 10 weeks before sowing, it was found that the inception of heading was greatly speeded up (Lojkin, 1936). At 16-22° C. and in a day-length of 15-16 hours plants from untreated seeds required about 150 days to reach the heading stage. For treated seeds the total period from the time of beginning the cold treatment to heading was about 110-120 days. Such treatments are now generally referred to as the *vernalization* of seeds.

With the aid of such treatments winter cereals can be planted in the spring and will produce grain the same growing season. The practical importance of this phenomenon, which is an excellent example of physiological preconditioning, has been stressed in recent years by Lyssenko and other Russian workers. Recent investigations have shown that the seeds of a number of other species besides winter wheat can also be vernalized.

Thompson (1933) has shown that the reproductive development of certain vegetable crop plants can be greatly influenced by pre-treatments at different temperatures. This type of physiological preconditioning is therefore in some respects similar to the process of vernalization. For example, when celery plants which had been grown in a greenhouse (60-70° F.) from the middle of February until April 1 were exposed for 30 days to a temperature of 40-50° F., seed stalks were produced by 74 per cent of the plants after transplanting into the field. In another group of plants from the same planting, set in the field at the same time, but not given a pre-treatment at a lower temperature, not a single seed stalk was produced.

Discussion Questions

1. In critical physiological experiments it is often desirable to work with a number of plants of identical genetic constitution. How can this be accomplished?
2. Tobacco leaves for cigar wrappers are grown in Connecticut under canvas shades. Why are larger leaves developed under these conditions than when the plants are grown in full sunlight?
3. Flax plants are grown for fiber in moist regions in which days are long, and often cloudy. When grown for oil the plants are grown in drier regions in which there is much clear, bright weather. What explanations can you suggest?
4. Why are north-facing slopes preferred to south-facing slopes as sites for orchards?

5. It is common nursery practice to cover the trunks of young trees with burlap or straw during the winter months. Can you see any benefits to the plants from this practice?
6. Peach trees often show winter injury on the side of the trunk with a southern exposure. The north sides of the trunk seldom show such injury. Explain.
7. It is a common belief in some regions that frozen plant tissues may often be revived without permanent injury if they are sprinkled generously with cold water. Is there any basis for such a belief?
8. With most floricultural plants which is the more practical way of increasing flowering during the winter months—increasing the intensity of light or its daily duration. Explain.
9. The critical light period for Mandarin soy beam is 17 hours, but it is a short-day plant. How would its behavior at the latitude of Washington (about 39° N.) and that of southern Canada (50-55° N.) differ?
10. Why do radishes rapidly "go to seed" if planted in late spring?
11. Some of the largest yields per acre of hay have been obtained in Alaska. Why is the climate of Alaska favorable for such crops?
12. In regrading lawns soil is often piled several feet deep over the roots of trees. Some species die following such treatment. Why? If a small area around the tree trunk is kept free from soil the trees may survive. Why?
13. What type of low temperature injury is probably most effective in preventing the natural spread of sub-tropical species into temperate regions?
14. Suggest reasons why northern species of plants often fail to survive when transplanted to more southern latitudes.
15. If a trench several feet deep is dug around an area of a few square rods in a forest and the trench then refilled with soil, herbaceous vegetation within this area often shows a better development than in surrounding untrenched areas, but sometimes does not. Suggest reasons for the differences in results.
16. List the important physiological processes occurring in a meristematic shoot of an herbaceous plant that will usually show a change in rate when a cloud passes across the sun after a period of direct exposure to sunlight on otherwise "standard day" conditions. Explain, for each process, whether you would expect an increase or decrease in rate and why.

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CHAPTER XXXIV

GROWTH CORRELATIONS

The development of any one organ of a plant is often influenced by the physiological processes or physico-chemical conditions prevailing in other organs of the same plant. In short, the shoot is part of the "environment" of the root, which in turn is part of the "environment" of the shoot, similar relationships, often reciprocal, existing between other organs of a plant. Such influences of one organ upon the development of another organ of the same plant are termed *growth correlations* or often simply *correlations*.

Growth correlations are not only exerted from one organ to another but also between tissues and between cells. The harmonious development of the plant body as a whole is controlled by correlative influences operating reciprocally from organ to organ, tissue to tissue, and cell to cell. The discussion in this chapter will be restricted almost entirely, however, to some of the better known examples of the correlative influences of one plant organ upon another.

Not all growth correlations are due to the same internal mechanism. Some influences of one plant organ upon another result from the effect of the first organ upon the distribution of one or more kinds of foods to other organs. Other correlations are due to the influence of the utilization of water by one plant organ upon the internal water relations of other organs (Chap. XVIII). Still others result from the influence of one organ of a plant upon the distribution of essential mineral elements to other organs. Many growth correlations are apparently due to the activity of hormones or hormone-like substances. Growth hormones which are synthesized in one organ are transported to other organs, often profoundly modifying the growth behavior of the recipient organs. Examples of such correlations have been described in the discussion of photoperiodism in the preceding chapter. Differences in electrical potential from one part of a plant to another may also play a rôle in the correlative development of plants.

Correlations between Reproductive and Vegetative Development.—A study of the correlation between vegetative development and fruiting in the tomato plant has been made by Murneek (1925). When tomato plants were deflorated or the fruits were removed as rapidly as they "set" the plants

continued to grow vegetatively. If, however, the fruits were allowed to remain on the plant and enlarge, vegetative development and the production of flowers gradually slowed down as more and more fruits began to develop. The steps in the inhibition of the development of such plants proceeded in approximately the following order: (1) loss of fecundity by the blossoms, (2) decrease in the size of the floral clusters, (3) abscission of the flower buds, (4) checking and later cessation of terminal growth of the stem and (5) eventual death of all parts of the plant except the fruit.

The checking effect of the enlargement of fruits upon continued vegetative development and the development of flowers resulted, according to this investigator, from the virtually complete monopolization of all of the nitrogen in the plants by the fruits. Carbohydrates, on the other hand, were found to be stored in considerable quantities in both the fruits and vegetative organs. In general, the more nitrogenous compounds available the more fruits that set and started to develop before inhibition of flowering and vegetative growth began. Removal of the fruits at any time before the vegetative parts died resulted in a renewal of vegetative growth, and, ultimately, in another cycle of reproductive development.

Development of flowers often has a checking effect on vegetative growth, while at a later stage in the life history of the same plant, fruiting may inhibit both further flowering and vegetative growth. This type of correlative development is shown by cotton plants (Pearsall, 1923) and many other species.

Correlative effects between fruiting and flowering can be observed in most species which develop flower primordia over a considerable period of time, as is true of many summer-blooming species. If the blossoms of the sweet pea (*Lathyrus odoratus*) are allowed to develop, for example, flowering soon ceases, but if they are picked from time to time flower primordia and blossoms are produced continually throughout the growing season. All experienced flower gardeners know that if continued flowering is to be maintained in many species, especially annuals, that the flowers must be cut as rapidly as they open, and that allowing fruit development to proceed soon results in a checking or even complete cessation of flowering.

It is usually considered that all of the growth correlations just described can be explained in terms of the internal food relations of plants. In general, they are believed to result from a diversion of such a large proportion of the available foods to developing flowers or fruits that other organs suffer a deficiency and hence are checked in growth. Both developing flowers and fruits are organs of high assimilatory and respiratory activity and hence their maturation may result in a considerable drain on the available food supply.

Some such correlative effects seem to be due to a virtual monopolization of nitrogenous foods by the dominant organ (*cf.* Murneek's results on tomato); others may be due mainly to the diversion of carbohydrate foods to the developing flowers or fruits.

The Shoot-Root Ratio.—A number of investigations have been made of the so-called shoot-root ratios in crop plants. Such ratios are usually calculated by dividing the dry weight of shoots by the dry weight of the roots produced during the growth period under consideration. The shoot-root ratio is influenced by reciprocal correlative influences between the aerial parts of a plant and its roots. The kind and magnitude of these correlative effects depend largely upon the environmental conditions to which the plant is exposed. For example, the nitrate concentration of the substratum has been shown to have a marked influence upon the shoot-root ratios of plants (Table 58).

TABLE 58—INFLUENCE OF NITRATE CONCENTRATION UPON THE SHOOT-ROOT RATIO OF BARLEY PLANTS. DURATION OF THE EXPERIMENT 49 DAYS (DATA OF TURNER, 1922)

	Dry weight of shoot in grams	Dry weight of roots in grams	S/R ratio
Low nitrate	9.64	1.81	5.33
Medium nitrate	11.81	1.43	8.28
High nitrate	10.55	1.17	9.08

The results of this experiment indicate a consistent increase in the shoot-root ratio with increase in the nitrate concentration of the solution culture. In this particular experiment there was also an absolute reduction in the dry weight of the roots developed with increase in nitrate concentration, but this was not found to be true in all the experiments performed by this investigator. Similar results have been obtained with a number of other species, and by plants rooted in the soil as well as in solution cultures.

The effect of nitrates upon the shoot-root ratio can be interpreted in terms of their influence upon the internal food relations of plants. If the nitrate concentration of the substratum in which the plant is rooted is low, most of the nitrates absorbed are utilized in the synthesis of amino acids in the roots, the carbohydrates necessary for this process being translocated downwards from the leaves. Most of these amino acids are used in the synthesis of protoplasmic proteins during the growth of the roots. Only a small proportion of the available nitrogenous compounds escapes utilization in the roots and is translocated (either as nitrates or as amino acids and related com-

pounds) to the aerial portions of the plant. The tops are therefore relatively deficient in proteins. Hence the growth rate of the aerial portions of the plant will be relatively slow and the shoot-root ratio relatively low.

When the supply of nitrates is more abundant, however, a smaller proportion of the total quantity absorbed is utilized in the roots. A larger proportion of the nitrogen, in one form or another, is translocated into the aerial portions of the plant, where much or all of it is usually utilized in the synthesis of protoplasmic proteins. The enhanced vegetative development of the aerial organs of the plant which is favored by such metabolic conditions results in the consumption of more carbohydrates as well as more proteinaceous foods by the aerial meristems. Because of the vigorous vegetative development of the shoot system the proportion of the carbohydrate foods which are translocated to the roots may be relatively small. Hence, relative to the shoots, the roots are likely to be deficient in both carbohydrate and protein foods, since synthesis of the latter requires carbohydrates as well as nitrates, and will grow at a relatively slower rate than the tops. The net result will be a higher shoot-root ratio than when the plants are grown in a soil which is deficient in nitrates.

Similarly, a decrease in the supply of carbohydrates within the plant, due to diminution in the rate of photosynthesis, or any other cause, influences the shoot-root ratio of plants. In general, diminution in the quantity of carbohydrate foods available in the tops results in an increased shoot-root ratio, and *vice versa*. Plants grown in the shade, for example, have higher shoot-root ratios than other plants of the same species grown in full sunlight. Similarly, pruning commonly results in increasing the shoot-root ratio of woody plants, since the new growth following pruning is usually especially vigorous, resulting in monopolization of most of the available carbohydrates by the shoots. The explanation of such effects follows a line of reasoning similar to that just presented in explanation of the relative influence of high and low nitrate supply on the shoot-root ratios of plants.

The shoot-root ratio is also influenced by the available soil water content. In general, a relatively low soil water content and adequate soil aeration favor relatively low shoot-root ratios, while the opposite conditions favor relatively high ones (Table 59).

The shoot-root ratios were computed in this investigation on a fresh weight basis but undoubtedly would show essentially the same relations if expressed on a dry weight basis. The results indicate clearly that the shoot-root ratio increases with increase in the percentage of water in the soil. The absolute weight of the shoots increases consistently with increase in soil water content, while the absolute weight of the roots increases to a maximum at a soil water

content of 20 per cent, after which it diminishes. An interpretation of the physiological basis of these results is left to the student as a problem.

TABLE 59—SHOOT-ROOT RATIOS OF CORN SEEDLINGS GROWN FOR 17 DAYS IN SAND AT VARIOUS WATER CONTENTS (DATA OF HARRIS, 1914)

Per cent water in terms of dry weight of sand	Fresh weight in grams		S/R ratio
	Shoots	Roots	
38	3.63	4.05	0.90
30	3.54	4.21	0.84
20	3.36	5.18	0.65
15	2.35	4.90	0.48
11	1.56	4.30	0.36

Certain mineral elements, especially phosphorus, also have effects on the shoot-root ratios of plants. It is also highly probable that the shoot-root ratio is influenced by the movement of hormone-like substances from shoot to root and *vice versa* (Chap. XXXII).

The principle of multiple causation (Chap. XXXIII) is especially well exemplified by the manner in which a similar end result, in terms of the shoot-root ratio, is brought about by various environmental conditions.

Apical Dominance.—In many herbaceous plants which produce aerial stems growth takes place principally or entirely at the apex of the main axis of the plant. Although a lateral bud is present in the axil of every leaf, side branches do not often develop from these buds as long as the terminal bud retains its vigor and continues to grow. If, however, the terminal bud is destroyed or injured in any way, or is artificially removed, development of one or more of the lateral buds usually starts at once. This inhibiting effect of a terminal bud upon lateral bud development is called *apical dominance* and is much more pronounced in some species than in others.

The phenomenon of apical dominance is usually also in evidence in all woody plants which produce true terminal buds. The lateral buds on current shoots usually do not develop unless the terminal bud is destroyed or injured. Development of the lateral buds on older shoot segments is of more frequent occurrence, indicating that the inhibitory effect of the apical bud diminishes with greater distance of the lateral buds from the apex of the stem. In many woody species most of the axillary buds regularly produce lateral branches the next growing season after the one during which they were produced.

The effect of the apical bud in apical dominance is apparently due to its auxin content. When agar blocks containing either auxin *b* or hetero-

auxin were applied to broad bean (*Vicia faba*) plants in place of the terminal buds which had been removed, the blocks being replaced with fresh ones from time to time in order to maintain the supply of auxin, inhibition of lateral bud development occurred just as if the terminal bud were intact (Skoog and Thimann, 1934). The lateral buds on check plants, to which only plain agar blocks were applied, developed rapidly. These investigators postulate that an excess of auxin is produced by the terminal bud, and that some of this auxin passes downwards into the lateral buds, exerting a direct retarding effect on the development of the latter. This explanation implies that the terminal buds will grow at auxin concentrations which are inhibitory to lateral buds.

A somewhat different interpretation of the mechanism of apical dominance has been advanced by Snow (1937) and others. The auxin moving downward from the terminal bud is supposed to favor growth of the stem through which it passes, and inhibition of the axillary buds is supposed to result from some secondary influence which arises as a result of the growth process.

Still another interpretation of this phenomenon is offered by Went (1938). He postulates that a hormone *caulocaline* (Chap. XXXII), necessary for stem elongation, moves upwards in stems and accumulates near the regions of auxin production. As long as the apical bud remains intact and produces auxin, practically all caulocaline is considered to move to the tissues in its vicinity and the lateral buds fail to develop. Inhibition of lateral bud development will continue if auxin is supplied artificially at the cut surface as described above. But if the apical bud is removed, or for some reason ceases to produce auxin, it is believed that the caulocaline is diverted to the lateral buds of lower auxin content and they begin to grow.

A somewhat similar example of correlative growth can be observed in many gymnosperms. The usual growth habit of most coniferous species, exhibited most rigorously by the spruces and firs, is for the main stem to grow vertically, while all of the lateral branches assume an obliquely upright or almost horizontal position. If, however, the apex of the terminal branch is destroyed or seriously injured in any way one or more (often all) of the lateral branches originating at the node next below the tip gradually turn up as a result of greater growth on their lower than on their upper sides. Eventually these branches assume an approximately vertical position, often imparting a candelabrum-shaped top to the tree. Subsequent vertical growth of the tree is accomplished by means of these reoriented branches. Maintenance of the more or less horizontal growth of the lateral branches in uninjured trees is obviously a result of some kind of a control exerted by the apical growing region. While no concrete evidence exists in favor of such

a theory the probability seems very great that this type of growth correlation is also to be explained in terms of a hormonal mechanism.

Polarity.—Many growth correlations are polar; that is, the two ends of a growing axis exhibit a marked dimorphism in development. The most familiar example of polarity in plants is that shown by cuttings, in which roots develop from the basal end and shoots from the apical end. Even if such cuttings are inverted and kept in a moist atmosphere roots will usually develop only from the morphologically basal end and shoots only from the morphologically apical end. In general, it is difficult to modify the inherent polarity of plant organs, although some exceptions to this statement are known.

While the obvious manifestations of polarity are morphological, basically all such phenomena depend upon a physiological mechanism. Many of the polar phenomena of plants are probably due to the polar transport of auxins or other hormones. The polarity of cuttings (Chap. XXXII) apparently can be explained largely, if not entirely, on a hormonal basis.

In addition to morphological polarities, and polarities in the distribution of certain compounds, plants also exhibit electrical polarities. The apices of the stems, hypocotyls, and coleoptiles of a number of herbaceous species have been shown to be electronegative relative to more basal portions (Clark, 1937). In larger plants, such as trees, the distribution of gradients of electrical potential are more complex (Lund, 1931). Apparently each individual cell in a plant is electrically polarized and acts like a tiny battery. The electrical potentials occurring in plant tissues are summation effects of the potentials of individual cells which may act either in series or in parallel (Rosene, 1935).

As a result of differences in electrical potential from one part of a plant to another electric currents flow continuously along certain circuits in the plant, and it has been suggested that these may serve as a mechanism of correlation. Electrical energy may thus be transferred from one cell to another, influencing the processes or development of the recipient cell. Although there is no doubt that polarity potentials exist in plants, the evidence that they are the basis of a correlation mechanism is as yet very insubstantial. On the other hand, it is possible that the differences in electrical potentials known to exist in plants are purely secondary phenomena which bear no casual relation to the correlative behavior of plants.

Discussion Questions

1. What environmental conditions lead to an increase in the shoot-root ratio? Which will lead to a decrease? Explain the probable mechanism of the action of each factor.

2. Why is it recommended that flowers be removed from Azaleas as soon as they have faded in order to insure abundant flowering the next year? Why is this practice more important in this species than in some others?
3. When the terminal meristem of many herbaceous plants (examples: many mints and asters) differentiates into an inflorescence development of lateral buds, previously dormant, often begins. What are some possible explanations?
4. Cotton plants continue to bloom throughout the summer yet few of the later flowers give rise to cotton bolls. Explain.
5. Commercial growers sometimes remove many of the young fruits from peach trees. This reduces the number of fruits in the resulting harvest yet may prove more profitable. Explain.
6. How can the development of differences of electrical potential in plant tissues be accounted for?

Suggested for Collateral Reading

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CHAPTER XXXV

GERMINATION AND DORMANCY

The Structure of Seeds.—All seeds contain an embryo plant which is enclosed by one, or more commonly by two, seed coats.[1] The seed coats originate from the integuments of the ovule and often exhibit external structural evidences of this origin even in the mature seed. Among these are the *hilum,* which represents the place where the seed was attached to the ovule stalk (*funiculus*), and *micropyle,* which frequently persists in the mature seed, and the *raphe,* a remnant of the ovule stalk which in certain kinds of seeds is adherent to the seed coats. When a single seed coat is present it is usually hard and woody but when there are two seed coats the inner is almost invariably thin and membranous.

The embryos in seeds of different species of plants differ markedly in size and appearance but in every case a mature embryo possesses one or more *cotyledons, a plumule,* and a *hypocotyl* (Fig. 137). The cotyledons are the seed leaves and they vary in number from one in the monocots to as many as fifteen in the embryos of some conifers. The embryos of dicots have two cotyledons, as the name implies. Structurally the cotyledons are modified leaves but usually they differ greatly in appearance from the foliage leaves of the same species. The cotyledons (or cotyledon) are attached near the upper end of the short thick stem-like axis of the embryo, the hypocotyl. The plumule or bud of the embryo is usually located just above the point at which the cotyledon or cotyledons are attached to the hypocotyl. The plumule consists of a meristem with several rudimentary foliage leaves. The primary root of the plant develops from the lower end of the hypocotyl. The rudimentary root at the lower end of the hypocotyl is often called the *radicle.*

An *endosperm* is also present in the seeds of many species (Fig. 137, *D*). This tissue develops from the endosperm nucleus and usually contains considerable quantities of accumulated foods. In the seeds of those species which contain no endosperm, such as the legumes, the cotyledons are usually en-

[1] In the skunk cabbage (*Spathyema foetida*) and possibly in a few other species the seed consists only of a naked embryo (Rosendahl, 1909).

larged and contain considerable quantities of reserve foods (Fig. 137, *B*). Some seeds contain a *perisperm* which represents remnants of the nucellus. The so-called "endosperm" in the seeds of gymnosperms is not a true endosperm, but represents the female gametophyte (Fig. 137, *E*).

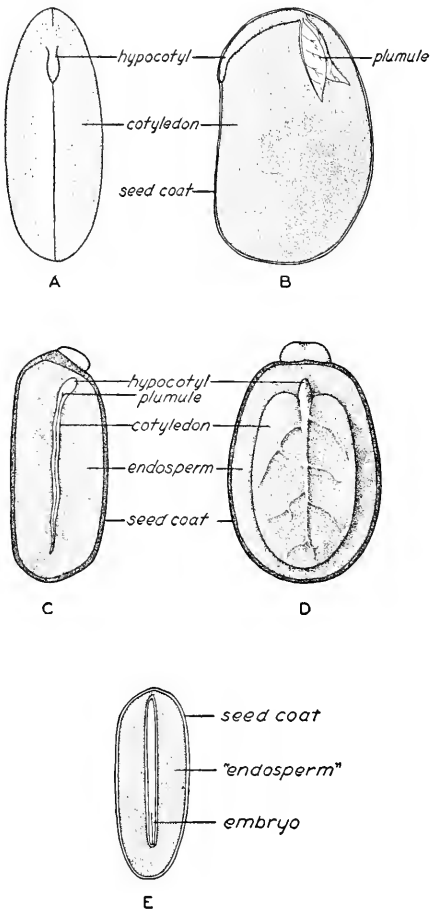

FIG. 137. Structure of seeds. (*A*) seed of lima bean with the seed coat removed, (*B*) seed of lima bean with one cotyledon removed, (*C*) seed of castor bean as seen in longitudinal median section, (*D*) seed of castor bean with endosperm removed down to the first cotyledon, (*E*) seed of pinyon (*Pinus edulis*) as seen in longitudinal median section.

Germination of Seeds.—The resumption of active growth on the part of the embryo resulting in the rupture of the seed coats and the emergence of the young plant is known as germination. The seeds of many plants will germinate as soon as ripe if environmental conditions are suitable. Pea seeds, for example, sometimes germinate within the pod, corn grains may sprout while still attached to the parent plant and the seeds of some citrus species frequently germinate while still within the fleshy fruits. Seeds of many other species, however, will not sprout until after an interval of weeks, months, or years, even if environmental conditions are favorable for germination. The causes of this condition of *dormancy* in seeds will be discussed later.

In nature, germination of seeds usually occurs either at or just below the surface of the soil. The latter is more apt to happen in forests where seeds, especially smaller varieties, often fall into interstices in the decaying detritus which composes the top layer of many forest soils, and are later covered by falling leaves. In the laboratory, non-dormant seeds will usually sprout if

they are brought into contact with any moist substratum or even simply exposed to a saturated atmosphere, provided other environmental conditions are also suitable.

The initial step in germination is the imbibition of water by the various tissues within the seed. This generally results in an increase in its volume. The increase in the hydration of the seed coats usually causes a pronounced increase in their permeability to oxygen and carbon dioxide, which is very low in the dry seed coats. The swelling of the seed often ruptures the seed coat, but in some species this does not occur until the emergence of the primary root.

With an increase in the hydration of the cells, enzymes become activated and zymogens are converted into enzymes. In seeds possessing an endosperm, enzymes apparently move into that tissue from the embryo as described in Chap. XXVII. Stored foods, whether they occur in the endosperm or cotyledons, are digested and the soluble products of the digestion process are translocated towards the growing points of the embryo. If chemical analyses are made of samples of seeds at successive stages during their germination it is found that the quantity of starches, oils, and proteins in the seed decreases markedly (Table 37). A large proportion of the fats present are usually converted, after digestion, into soluble carbohydrates. The soluble carbohydrates are not present during the later stages of germination in amounts quantitatively equivalent to the starch or other storage carbohydrates digested during the process, indicating that a large proportion of these compounds is consumed in respiration or assimilated in the construction of the carbohydrate constituents of cell walls. In oily seeds the soluble carbohydrates utilized in respiration result largely from chemical transformations of the products of the digestion of fats. Digested proteins are usually represented in the seeds by quantitatively equivalent amounts of amino acids, asparagine, etc. This indicates that proteins are not consumed in respiration but are utilized in the synthesis of the organic nitrogen compounds of the growing embryo.

Insofar as the actual mechanics of seed germination are concerned two principal groups of seeds may be recognized: (1) those in which the cotyledons emerge from the seed and (2) those in which the cotyledons remain permanently within the seed. Most seeds of dicots and seeds of some monocots such as onion belong to the first group while the seeds of grasses and of some dicots such as peas and oaks belong in the second.

1. *Seeds in Which the Cotyledons Emerge.*—The sequence of events that takes place during the germination of the seed of the lima bean (*Phaseolus lunatus*) will be described as a type example of this group (Fig. 138). Germination is initiated by a marked swelling of the seed which usually rup-

tures the seed coat. This is followed by the emergence of the primary root which develops from the lower end of the hypocotyl and is the first structure of the embryo to make contact with the external environment. The primary root grows downward in the soil producing lateral roots and root hairs. The hypocotyl then elongates rapidly, pulling the cotyledons upward out of the soil into the air where they separate into an approximately horizontal position on either side of the plumule. The plumule then begins active growth giving rise to the stem and foliage leaves of the seedling. Since the bean is a seed without an endosperm the food used during germination is largely derived from the accumulations in the thick cotyledons.

FIG. 138. Stages in the germination of a seed of lima bean (*Phaseolus lunatus*).

2. *Seeds in Which the Cotyledons Do Not Emerge.*—The seed of the pea is structurally very similar to that of the bean but its germination behavior is very different. Elongation of the hypocotyl does not occur and the cotyledons remain in the seed. The primary root elongates early in the process of germination much as in the bean. The plumule is elevated through the soil by rapid elongation of the *epicotyl,* which is the stem region between the cotyledons and the first true leaves—in other words the first internode.

This type of germination is also exhibited by oak acorns (Fig. 139).

Many monocots also show this type of germination behavior. In the germination of the corn grain, for example, the primary root develops from

the lower end of the axis of the embryo, growing through the *colcorhiza* (Fig. 103) and the wall of the grain (pericarp). As the primary root elon-

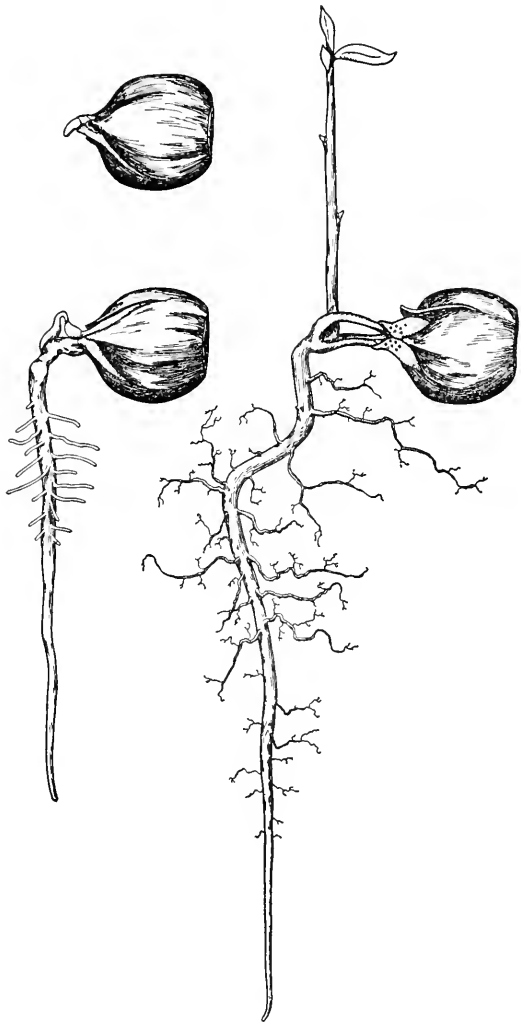

FIG. 139. Stages in the germination of a red oak (*Quercus borealis*) acorn. Redrawn from Korstian (1927).

gates lateral roots soon appear and root hairs begin to develop just back of the elongating regions on all of the roots. The single cotyledon (*scutellum*)

remains within the seed and acts as an absorbing organ through which soluble foods in the endosperm move into the tissues of the rapidly enlarging embryo. Soon after the appearance of the primary root the plumule and the *coleoptile,* which completely encloses it, grow out through the walls of the grain and upward as a result of elongation of the region of the axis just below the plumule. About the time the coleoptile breaks through the surface of the soil, or soon thereafter, the first foliage leaf grows through the tip of the coleoptile and emerges into the light and air.

Environmental Conditions Necessary for Germination.—The seeds of all species of plants require at least three external conditions before germination can occur: (1) water, (2) a suitable temperature, and (3) oxygen. A fourth factor, light, appears to be essential for the germination of the seeds of a few species and to influence the germination of seeds of some other species.

1. *Water.*—A low water content is one of the prominent characteristics of resting seeds and since physiological processes occur largely in an aqueous medium, germination cannot occur unless the seed can absorb water from its environment. The absorption of water by seeds initiates the series of physical and chemical processes which, in the absence of any limiting factor, results in the emergence of the embryo from the seed. In general, however, complete germination of seeds will not occur in a soil with a water content below the wilting percentage (Chap. XVI).

Water-vapor as well as liquid water can be imbibed by seeds. Most seeds will therefore pass through the earlier stages of germination in an atmosphere which is saturated, or nearly so, but if the vapor pressure of the atmosphere is appreciably below the saturation value germination will be checked or inhibited.

2. *Oxygen.*—The respiration of germinating seeds proceeds at a rapid rate especially during the early stages of germination. The partial pressure of oxygen in the atmosphere can be considerably reduced, however, without greatly interfering with the rate of respiration (Chap. XXIX). In fact the seeds of some water plants such as cat-tail (*Typha latifolia*) germinate better under low oxygen pressures than in air. Seeds of many terrestrial plants can germinate under water where the concentration of oxygen often corresponds to a partial pressure of oxygen very much less than that of the atmosphere (Morinaga, 1926). It is probable that seeds buried deeply in compact soils are often prevented from germination by the very low partial pressure of oxygen in such an environment. During the early stages of germination of seeds of pea and some other species respiration is largely or almost entirely of the anaerobic type because of the relative impermeability of even the hydrated seed coats of such species to oxygen (Chap. XXX). As soon as

the seed coats are ruptured, however, aerobic respiration replaces anaerobic oxidative processes even in seeds of this type.

3. *Suitable Temperature.*—In the absence of other limiting factors the seeds of any species will germinate within a certain range of temperatures, but at temperatures above or below this range no germination will occur. As a rule, the seeds of species indigenous to temperate regions germinate in a lower range of temperatures than seeds of species whose native habitat is in tropical or subtropical regions. Wheat seeds, for example, germinate at temperatures only slightly above 0° C. and at temperatures as high as 35° C., while the range of temperatures for germination of seeds of maize (a species of subtropical origin) lies between a lower value of 5-10° C. and an upper limit of about 45° C. The optimum temperature is usually about midway between the two extremes of temperature at which germination will occur. It is not possible to designate any exact temperature as the optimum for germination as this varies with the other prevailing environmental conditions and also with the exact criterion selected as an index of germination. The most favorable temperature for the elongation of the primary root, for example, does not always correspond to the most suitable temperature for the development of the plumule.

4. *Light.*—A few species of plants including the strangling fig (*Ficus aurea*), mistletoe (*Viscum album*) and some other epiphytes, have seeds which fail to germinate unless exposed to the light. Many kinds of seeds germinate better when exposed to the light than when kept in total darkness. Examples of these light-favored seeds are those of many grasses, especially species of *Poa,* the evening primrose (*Oenethera biennis*) and mullein (*Verbascum thapsus*). On the other hand the germination of the seeds of some species appears to be retarded or even prevented by exposure to light. Seeds of the onion and many other members of the lily family belong in this group (Crocker, 1936). The effect of light upon the germination of seeds is profoundly influenced by other environmental factors. For example, the germination of the seeds of some species of grass (*Poa* sp.) is ordinarily influenced by light, but after a period of dry storage this effect disappears. Similarly the ordinarily light sensitive seeds of the pampas grass (*Chloris ciliata*) will germinate readily in complete darkness in an atmosphere of pure oxygen.

Dormancy of Seeds.—Many kinds of seeds, apparently ripe, fail to germinate even if placed under such conditions that all environmental factors are favorable. In such seeds resumption of growth by the embryo is arrested by conditions within the seeds themselves. The state of inhibited growth of seeds or other plant organs due to internal causes is usually called *dormancy,* but is sometimes referred to as the "rest period."

Failure of seeds to sprout does not necessarily mean that they are dormant. Environmental conditions may be unfavorable for germination. The water supply may be inadequate, or the temperature may be unfavorable. Deeply buried seeds are often prevented from germinating by an inadequate oxygen supply, or certain kinds of light-sensitive seeds may fail to germinate because of unfavorable light conditions. The term dormancy as applied to seeds is generally restricted to those which fail to germinate as a result of internal causes. For convenience we shall use the term *quiescence* as a designation for the situation in which failure of a plant organ to grow is due to environmental conditions. In practice it is often difficult to determine whether seeds or other plant organs under natural conditions are actually dormant or merely quiescent without resorting to an experimental test.

Dormancy of seeds is due to one or a combination of several different factors (Crocker, 1916):

1. *Seed Coats Impermeable to Water.*—The seed coats of many species are completely impermeable to water (and probably also to oxygen) at the time the seeds are ripe. This condition is very common in the seeds of many legumes (clovers, alfalfa, black locust, honey locust, etc.), of the water lotus, and of the morning glory. Germination fails to occur until water penetrates through the seed coats. In many such seeds the permeability of the coats to water increases slowly in dry storage but it occurs more rapidly when they are exposed to the fluctuations of temperature and moisture that are present in soils under natural conditions. The action of bacteria and fungi also increases the permeability of the seed coats to water and so shortens the dormant period of seeds of this kind that are buried in the surface layers of soil.

2. *Mechanically Resistant Seed Coats.*—The seeds of some of the commonest weeds such as mustard (*Brassica* sp.), pigweed (*Amaranthus* sp.), water plantain (*Alisma*), shepherd's purse (*Capsella*) and peppergrass (*Lepidium*), remain in the dormant condition because the seed coats are strong enough to prevent any appreciable expansion of the embryo. In the seeds of the pigweed (*Amaranthus retroflexus*), for example, water and oxygen penetrate through the seed coats readily, but the enlargement of the embryo is limited by the mechanical strength of the seed coats. As long as the seed coats remain saturated with water, dormancy persists, and may last for a period of thirty years or even longer. If the seed coats become dry, however, certain changes occur in the colloidal compounds that make up the walls of the cells of the seed coats so that upon being again saturated with water they are no longer able to resist the pressures developed by the imbibitional forces in the embryo. The coats are ruptured and germination occurs. High temperatures (above 40° C.) may also induce some germination of pigweed

seeds at the time of ripening since the seed coats are less resistant at these temperatures. As the seeds age, the minimum temperature for germination becomes lower. The embryos of these seeds have no dormant periods and will grow readily if the seed coats are removed. Likewise any treatment which weakens the seed coats increases the percentage of seeds that germinate. In general, other seeds with mechanically resistant seed coats exhibit similar behavior.

3. *Seed Coats Impermeable to Oxygen.*—The two seeds in a fruit of cocklebur (*Xanthium* sp.) are not equally dormant. Under natural conditions the lower seed usually germinates in the spring following maturity while the upper seed remains dormant until the next year. The dormancy of these seeds has been demonstrated to result from the impermeability of the seed coats to oxygen (Shull, 1911). If the seed coats are ruptured, or if the oxygen pressure is increased around intact seeds, germination occurs. The oxygen requirements for germination are greater in the upper seed than in the lower and this explains the more pronounced dormancy of the former. During dry storage or under natural conditions the seed coats gradually become more permeable to oxygen and the oxygen requirements of the embryo decrease. Hence the intensity of the dormant condition gradually diminishes. The seeds of a number of grasses and of many *Compositae* also have dormant periods that seem to be due to the impermeability of the seed coats to oxygen.

4. *Rudimentary Embryos.*—Many species of plants have seeds in which the embryo does not develop as rapidly as the surrounding tissues so that when the seeds are shed the embryos are still imperfectly developed. In some species the ripened seeds contain embryos that have grown little beyond the fertilized egg stage while in other species development of the embryos may be nearly complete when the seeds are shed. The germination of such seeds is necessarily delayed until formation of the embryo is complete. Examples of species in which dormancy of seeds is due to incompletely developed embryos include ginkgo (*Ginkgo biloba*), European ash (*Fraxinus excelsior*), holly (*Ilex opaca*) and many orchids.

5. *Dormant Embryos.*—In many species although the embryos are completely developed when the seed is ripe, the seeds fail to germinate even when environmental conditions are favorable. Dormancy of such seeds is due to the physiological condition of the embryo. The embryo of such seeds will not grow when the seeds first ripen even if the seed coats are removed. Among the many species whose seeds exhibit dormancy of this type are apple, peach, hawthorne, iris, lily-of-the-valley, basswood, ashes, tulip poplar, dogwood, hemlock, and pines. Germination of such seeds occurs only after a period of "after-ripening." In many wild species after-ripening occurs

during the winter while the seeds lie on the ground or just under the soil surface. Such seeds will not germinate in the fall just after they are shed, but will germinate the following spring if environmental conditions are favorable. In some species after-ripening occurs over a period of years, some germination occurring each year. After-ripening involves principally a series of changes in the physiological condition of the embryo which gradually converts a dormant embryo into one which can resume growth. The nature of these physiological changes is not clearly understood at the present time. In some species after-ripening also involves changes in the properties of the seed coats. The length of time required for completion of the after-ripening process can be greatly modified by environmental conditions as subsequent discussion will show.

Secondary Dormancy.—Some seeds which are capable of germinating as soon as they are harvested lose this capacity after being kept in an unfavorable environment for a while. This induced dormant period is known as *secondary dormancy*. Usually secondary dormancy can develop only when at least one of the conditions essential for germination is unfavorable. For example, if seeds of white mustard (*Brassica alba*) are exposed to high concentrations of carbon dioxide they fail to germinate, even under favorable conditions, for a long period after the removal of the carbon dioxide (Kidd and West, 1917). Light sensitive seeds may pass into secondary dormancy if kept in the dark and seeds that germinate only in the dark may become dormant if exposed to light. Likewise secondary dormancy may be induced in some kinds of seeds by exposures to low temperatures and in others by high temperatures (Davis, 1930).

Secondary dormancy is often caused by changes in the seed coats since in some species the embryos are able to grow immediately if the seed coats are removed. In other kinds of seeds, however, the dormancy is produced by physiological changes that occur within the embryo itself. Secondary dormancy, like primary dormancy, may be interrupted by various treatments.

Methods of Breaking the Dormancy of Seeds.—The dormancy of seeds presents a practical problem of considerable economic importance. Plant growers are often interested in securing seed which will germinate soon after it is harvested. Ordinarily this would be possible only with seeds which have a short dormant period or none at all. Methods have been devised, however, whereby the dormancy of many kinds of seeds can be broken, and whereby the length of the dormant period in many other kinds can be shortened. The methods employed for the breaking of dormancy vary depending upon its cause. Methods which can be used for breaking the dormancy of one species may be

totally ineffective when used with seeds of another species, and sometimes may even prolong dormancy.

1. *Scarification.*—Whenever dormancy is due to any of the causes inherent in the seed coats it can be interrupted by scarification. We shall use this term to apply to any treatment—mechanical or otherwise—which results in rupturing or weakening the seed coats sufficiently to permit germination. For example, machine-threshed legume seeds usually show a higher percentage of germination than those that have been harvested by hand. The mechanical treatment is sufficiently severe to scratch or crack many of the seed coats and this permits ready ingress of water. Various types of mechanical treatments have been devised for breaking the dormancy of seeds of this kind. Strong mineral acids have likewise been used successfully to interrupt seed dormancy caused by resistant or impermeable seed coats. It is essential, however, that any method used to interrupt seed coat dormancy should not be injurious to the embryo. Under natural conditions dormancy of such seeds is broken by the slow decay of the seed coats or by the action of alternate freezing and thawing.

2. *Low Temperatures.*—After-ripening of many seeds occurs more rapidly when they are stratified in moist peat at low temperatures than when stored at higher temperatures. Temperatures between 5° and 10° C. for two or three months are effective with conifer seeds (Barton, 1930) and greatly increase the percentage of germination. Similarly, low temperatures combined with moisture have been found to reduce the period of after-ripening in seeds of mountain ash, basswood, elder, bayberry and many other species. The effectiveness of low temperatures in breaking dormancy appears to be associated in some species at least with a favorable relation between respiration rates and the rate of oxygen absorption or carbon dioxide liberation. Permeability changes in the seed coats may also be an important factor.

3. *Alternating Temperatures.*—In some seed testing laboratories it is common practice to subject seeds alternately to relatively low and high temperatures. The temperature extremes of such treatments may not differ by more than 10° or 20° C. and both are commonly well above the freezing point. The germination of seeds of Kentucky blue grass (*Poa pratensis*), for example, is greatly improved by subjecting the seeds alternately to temperatures of 20° C. for 16-18 hours and 30° C. for 6-8 hours and the percentage germination of Johnson grass (*Holcus halepensis*) seeds is increased by alternate treatments at 30° C. for 18-22 hours and 45° C. for 2-8 hours (Harrington, 1923). The dormancy of some seeds may be interrupted by alternate freezing and thawing though this is decidedly harmful to other species. The action of the alternating temperatures upon the seeds is not

understood. It is entirely ineffective with seeds of some species. Seeds of carrot and timothy, for example, germinate just as well at constant temperatures as when temperatures are varied. In general this method of treatment is used principally with seeds in which dormancy is inherent in the embryo.

4. *Light.*—In the previous discussion light was mentioned as one of the conditions essential for the germination of certain species of seeds. Light may be considered, therefore, as a means of breaking the dormancy of such species. In some of the species other environmental factors can be substituted for light. In seeds of *Veronica longifolia* (one of the commonly cultivated speedwells), for example, light improves germination at low temperatures but the seeds germinate equally well in total darkness at high temperatures. In seeds of Kentucky blue grass exposure to light is effective in improving germination at both intermittent and constant temperatures. No satisfactory explanation of these facts has yet been suggested.

5. *Pressures.*—Seeds of sweet clover (*Melilotus alba*) and alfalfa (*Medicago sativa*) showed greatly improved germination after being subjected to hydraulic pressures of 2000 atmos. at 18° C. (Davies, 1928). When the pressure was applied for periods of from 5-20 minutes the germination of the seeds was increased by 50-200 per cent. The effect of the pressures persists after the seeds have been dried and stored and is undoubtedly due to changes in the permeability of the seed coats to water.

Longevity of Seeds.—The life-span of seeds varies from a few weeks to many years, depending upon the species and the environmental conditions to which the seeds are subjected. The silver maple (*Acer saccharinum*) may be cited as an example of a species which has short-lived seeds. When the seeds of this species are shed in June their water content is about 58 per cent. Once their moisture content drops below 30 to 34 per cent the seeds die (Jones, 1920). Since this often happens within a few weeks in nature, seeds of this species soon perish. The seeds of the majority of crop plants are relatively short-lived under the usual storage conditions, generally remaining viable for only one to three years. The life-span of such seeds can often be increased several fold by keeping them under suitable storage conditions.

At the other extreme there are a few authentic records of seeds which have lived for more than a hundred years. Bequerel (1934) succeeded in germinating in 1934 seeds of *Cassia bicapsularis* which had been collected in 1819, and seeds of *Cassia multijuga* which had been collected in 1776. These are both South American species of legumes. Viable seeds of the Indian lotus (*Nelumbo nucifera*) have been found buried under layers of peat and soil in Manchuria of such depth that they must have been at least 120

years old and may have been 200 to 400 years old (Ohga, 1927). With this one exception all the authentic records of seeds living for 75 years or longer are of legumes. Further data on the longevity of seeds are given by Crocker (1938).

At least some of the seeds of a number of species of wild plants will remain viable for 50 years or more. This is especially true of hard-coated species. As a general rule only seeds with a pronounced dormancy remain viable very many years in nature. The seeds of many weed species are notoriously long-lived as compared with the seeds of most crop plants. This is illustrated by an experiment initiated by Beal at East Lansing, Michigan in 1879. Seeds of twenty herbaceous species were mixed with sand and buried in pint bottles. Twenty such bottles were prepared. Once every five or ten years one of the bottles was excavated and the enclosed seeds tested for their percentage of germination. Fifty years later seeds of five species remained alive and showed the following percentages of germination: yellow dock (*Rumex crispus*) 52 per cent, evening primrose (*Oenethera biennis*) 38 per cent, moth mullein (*Verbascum blattaria*) 62 per cent, black mustard (*Brassica nigra*) 8 per cent, and water smartweed (*Polygonum hydropiper*) 4 per cent (Darlington, 1931).

Dormancy of Buds.—The buds of many species of plants also exhibit the phenomenon of dormancy. In spite of favorable environmental conditions which often prevail the buds on woody plants of temperate zones do not usually develop into shoots the same season they are formed. Some important exceptions to this statement are discussed in the next chapter. The length of time for which such buds retain their dormancy varies considerably according to species. Howard (1910) has made a comprehensive study of bud dormancy in a large number of species of woody plants, some of American, some of European, and some of Asiatic origin. This investigation was conducted in Missouri. Of 234 species collected, buds of 125 species developed when branches were brought into a greenhouse between October 28 and November 4. Most of these were European or Asiatic forms. In other words the buds of more than half of the species experimented with were not in a dormant state on this date. A second collection of branches from 283 species was brought into the greenhouse on January 8-10. Of these buds developed on 244 species. On February 23 a third collection of 63 species was made composed largely of kinds on which the buds failed to develop in the preceding two tests. On this date buds developed on all but five of the species collected. The results of this study indicate that the buds of some woody species retain their dormancy much longer into the fall or winter than others

and that the buds of many such species are in a quiescent rather than a dormant state during much of the winter.

Dormancy of buds is sometimes due to correlative effects, many of which probably have a hormonal mechanism. A familiar example is the phenomenon of apical dominance (Chap. XXXIV), in which lateral buds remain dormant as long as the terminal growing region remains intact, but usually resume growth upon its injury or removal.

Likewise the buds of many kinds of tubers, rhizomes, corms, and bulbs often remain dormant for periods of greater or less duration while environmental conditions are favorable for their development.

Methods of Breaking the Dormancy of Buds.—As the previous discussion has shown the buds on many species of woody plants remain dormant through the autumn and at least the forepart of the winter. This dormancy is not caused by low temperatures as it is also exhibited by such species when kept in a greenhouse where the temperature is continuously maintained at levels typical of midsummer weather. The dormant condition often persists as long as the temperatures are high but may be broken by subjecting the plants to temperatures near the freezing point (Coville, 1920). The buds of some species such as peach will begin to grow, if environmental conditions are favorable, after only a few days of chilling, but the buds of other kinds of plants such as the blueberry (*Vaccinium corymbosum*) fail to grow well unless exposed to temperatures near the freezing point for several weeks. Coville has demonstrated that the effect of the low temperatures is restricted to the tissues exposed. When single branches are chilled while the rest of the plant is kept warm, only the buds on the chilled branch are able to grow, all of the others remaining dormant (Fig. 140).

The dormant buds of many species of plants may be induced to grow by immersing them in warm water for several hours. Molisch, who discovered this method of forcing dormant buds, found that submerging the branches in a water bath at 30 to 35° C. for 9 to 12 hours was effective with many species. The success of the treatment varies with the kind of plant and with the time of the year at which the treatment is applied. Buds of some species fail to react to the warm bath treatment in September but grow readily if treated in the same way in October or November. Like the low temperature treatment just described the effect of the warm bath is local, only those buds that are brought into contact with the warm water developing.

Early in the Twentieth Century Johannsen discovered that dormant buds of many kinds of plants will begin to grow after being exposed to vapors of ether or chloroform for a day or two. The interval of time between the ether treatment and the beginning of growth differs widely with the time of the

year at which the vapor is applied. In late summer or early fall several weeks may elapse between the exposure to ether vapor and the active growth of the buds. Late in the winter, however, the interval is shortened to a day or two.

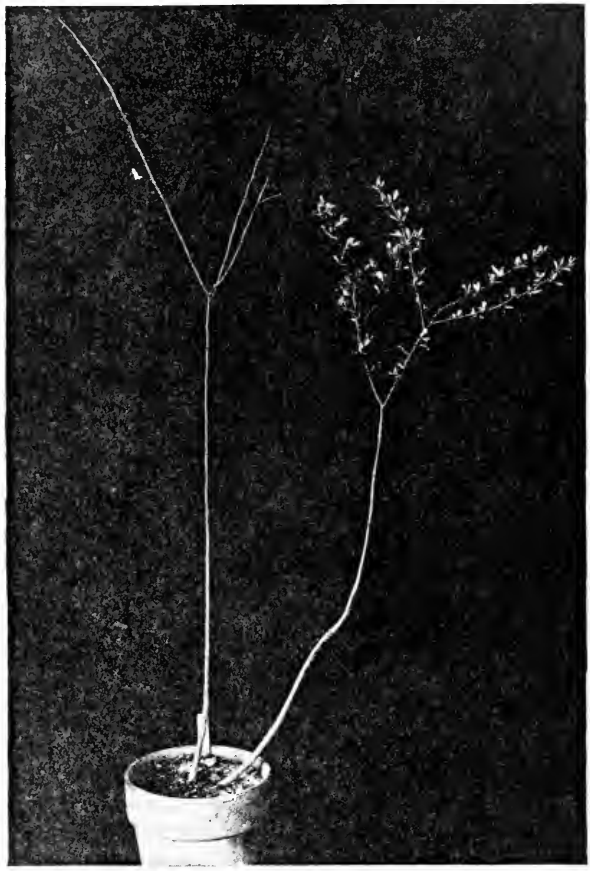

FIG. 140. Breaking dormancy of buds of blueberry by low temperatures. The branch on the right was exposed to low temperatures by allowing it to project through a small hole in a greenhouse during the winter. The branch on the left remained within the greenhouse. Figure shows appearance of plants on Apr. 18. Photograph from Coville (1920).

More recently a number of other chemical treatments have been discovered that are successful in breaking the dormancy of buds. Denny (1926) found that dormant potato tubers sprouted freely when exposed to ethylene chlorhydrin vapor. Sodium and potassium thiocyanate, thiourea, dichloroethylene,

carbon bisulfid, xylol, ethyl bromid, and a number of other compounds were also effective. Vapors of ethylene chlorhydrin so hastened the growth of tubers of the Irish cobbler variety that vines two feet high bearing young tubers 1 cm. in diameter grew from the treated tubers before the sprouts of the untreated tubers appeared above the surface of the ground. Solutions of sodium and potassium thiocyanate gave almost equally striking results. Thiourea solutions differed somewhat from the other compounds used in that

FIG. 141. Effect of ethylene dichlorid in breaking dormancy of lilac buds. Plant on right received no treatment. Plant on left exposed 48 hours to ethylene dichlorid, 2.5 cc. of liquid per 100 liters of space on Dec. 10. Both plants kept in greenhouse and photographed early in January. Photograph from Denny and Stanton (1928).

they overcame the inhibiting effect of the terminal bud upon the growth of lateral buds and caused the development of several shoots from each eye on the tuber.

The vapors of ethylene chlorhydrin and ethylene dichlorid also induce the growth of dormant buds of lilac (*Syringa vulgaris*), flowering almond (*Prunus triloba*), and some other species of woody plants (Fig. 141). The effect of the vapors was so restricted that when one of two paired buds of a lilac was exposed to the vapor, growth occurred only in the treated bud. The untreated bud remained as fully dormant as other buds more distant from

the treated area. Apparently the factors causing the dormancy of the buds of these plants reside within the buds themselves and not in the adjacent tissues.

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CHAPTER XXXVI

GROWTH PERIODICITY

The growth of a plant or plant organ never proceeds steadily hour after hour or day after day, but is subject to more or less regularly recurring, often rhythmical, daily and seasonal variations in rate. Seasonal variations in growth phenomena involve qualitative as well as quantitative differences in development during different stages of the growth cycle. Most plants, for example, produce flowers only at certain stages in their life history and either grow only vegetatively or not at all at other times. The more obvious examples of growth periodicity often correlate very closely with cyclical daily or seasonal variations in environmental conditions, but internal factors also play an important rôle in many periodic growth phenomena.

Daily Periodicity of Growth.—All actively growing plant organs characteristically exhibit a daily periodicity in growth rate. A number of studies have been made of daily variations in the rate of increase in the length of stems or monocot leaves. Elongation of either of these types of organs involves both the cell division and cell enlargement phases of growth. Under environmental conditions approximating those of a "standard day" (Chap. XIII) curves for the daily periodicity of elongation of plant organs will often approximate those shown in Fig. 142. The maximum rate of elongation of hyacinth leaves occurred in this experiment between midnight and 4 A. M. The rate of elongation then gradually diminished until a minimum value was reached a little after noon. Subsequently there was usually a consistent rise until the maximum value was again attained.

Cyclical variations in the rate of elongation of plant organs during the course of a day can be interpreted in terms of the principle of limiting factors. During the progress of the day first one factor and then another is limiting. The rate of growth at any particular moment will be largely limited by the factor in relative minimum at that time. The three principal factors influencing the daily periodicity in the rate of elongation of plant organs are the internal water relations of the plant, light, and temperature.

Under conditions which favor a daily periodicity of elongation of the type just described a water deficit usually develops within the plant, attain-

ing its maximum during the afternoon hours (Chap. XVIII). The gradual diminution in elongation rate during the daylight hours parallels more or less closely this decrease in the water content of the plant. Conversely the gradual acceleration in elongation rate which takes place during the hours of darkness reflects the increase in the hydration of the tissues which occurs during this period. Actual growth rates are influenced primarily by the variations in the hydration of the meristematic tissues but, in general, changes in the hydration of meristems parallel variations in the hydration of the plant as a whole. Since both cell division and cell enlargement are soon retarded by a deficiency of water in a plant, this close correlation between daily variations in rate of elongation and the intensity of the internal water deficit indicates clearly that the latter is an important factor in determining the daily periodicity of growth.

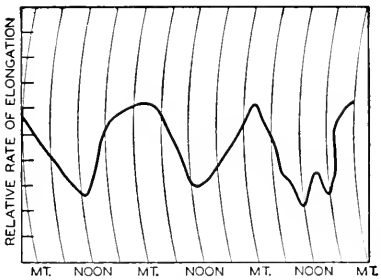

FIG. 142. Daily periodicity in rate of elongation of hyacinth leaves. Data of MacDougal (1901).

Light also influences the daily periodicity of elongation of plant organs, although it is uncertain whether its direct effects (Chap. XXXII) or its indirect heating effects are the more important. As previous discussion has shown, because of the higher temperature of the leaves the rate of water loss of plants exposed to a high light intensity is usually greater than that of plants exposed to a low light intensity under otherwise similar conditions. Consequently more severe internal water deficits usually develop under high light intensities than under low ones. Light therefore affects growth rates at least in part through its effect upon the internal water relations of the plant. The usual retardation in the rate of elongation of plant organs which occurs during the daylight hours of clear days is at least partly a result of either direct or indirect effects of light, and most probably of both.

During the night hours temperature is undoubtedly very often the factor limiting the rate of increase in length of plant organs. Conversely daytime temperatures may be higher than the optimum for elongation or enlargement under the other prevailing environmental conditions and may thus contribute to the retardation in rate of increase in length of plant organs which is of frequent occurrence during the daylight hours.

Innumerable other patterns of daily elongation periodicity are possible, depending upon the cyclical behavior of environmental and internal factors. Only a few of these will be mentioned. Other extreme factors being equal,

daytime rates of elongation are more rapid on cloudy than on clear days. In many species, including maize (Loomis, 1934) transit of a cloud across the sun permits a temporary acceleration in growth rate.

Under some conditions maximum rates of elongation of plant organs may occur in the daytime rather than at night. In the early spring, for example, low night temperatures may inhibit or greatly retard the elongation of the leaves of such species as wheat and blue grass which grow at this season, while elongation occurs at more rapid rates during the warmer daylight hours. Because of the retarding effect of the light factor such a daily periodicity of elongation is most likely to occur when relatively warm, cloudy days are accompanied by cool nights.

Seasonal Periodicity of Vegetative Growth.—All plants exhibit more or less clearly marked seasonal variations in the rate of vegetative growth.

The seasonal periodicity in the vegetative growth of any species is conditioned partly by environmental factors and partly by internal conditions. Among the former temperature and water supply are especially important. Some of the internal conditions which are known to play a significant part in such phenomena are dormancy, internal water relations, and correlative effects among organs. In temperate regions the periodic vernal resumption of growth by woody perennials is one of the most spectacular biological accompaniments of the march of the seasons. This topic will be discussed almost entirely in terms of such woody plants.

A discussion of the periodicity of growth in woody plants may logically begin with bud formation, which starts as an integral part of the resumption of growth in the spring. In most species development of buds is completed or at least well advanced by midsummer, although to the casual observer they do not ordinarily become conspicuous until defoliation of the twigs occurs in the autumn. Cell divisions in the apical meristems enclosed within the bud scales during the summer months result in the production of an embryonic shoot. In many, but by no means all, species no more leaves are borne on an annual shoot than have developed in rudimentary form in the bud during the preceding summer. Each bud on a woody plant therefore represents essentially an immature annual shoot.

Buds do not normally develop during the season they are produced but, with certain exceptions to be noted shortly, remain for some time in a dormant state. As shown in the preceding chapter the length of time that buds on woody plants remain dormant varies greatly according to species. Some lose their dormancy early in the autumn; others retain it until late in the winter. The buds of temperate zone woody plants seldom open as soon as they lose their dormancy but remain in a quiescent state until the favorable

environmental conditions of spring. Low temperature is probably the principal factor preventing development of quiescent buds in the late winter and early spring, although a deficient water supply may also be involved, at least in some species. Photoperiodic effects may also play a part in the vernal resumption of vegetative growth by plants.

The development of new shoots from the buds on woody stems in the spring is by no means always a continuous process. In species such as cherries and willows which "leaf out" relatively early, the growth process is often intermittent. During this season periods of warm weather often alternate with colder spells. Hence elongation of the developing shoots may take place in a series of short spurts, each terminated upon the advent of unfavorably cool weather. Apical growth is much more likely to proceed uninterruptedly in species such as beech and the hickories in which it is initiated later in the spring. Under favorable conditions practically all stem elongation and development of the new crop of leaves may occur in such species during a growth period lasting only two or three weeks. Termination of the spring burst of growth in such species is evidently due to internal causes, since environmental conditions usually remain favorable for growth during much or all of the summer.

The stems of some ligneous species (sumac, dogwood, ailanthus, etc.) do not grow in the definite manner described above, but continue to elongate, producing leaf after leaf, for most or all of the summer, quiescent intermissions occurring only when environmental conditions are unfavorable. In some such species growth is not terminated until the advent of frost.

Under some conditions the buds on woody stems open the same season they are formed. This is a commoner occurrence in some species than in others, and is more likely to happen on young trees or shrubs than on old ones. Defoliation of a tree relatively early in the season usually results in a resumption of growth from buds developed during the current season. During wet summers development of the currently produced buds into shoots occurs frequently in many species of woody plants. Such shoots are often called "Lammas-shoots."[1] Among the oaks, especially when young, the production of two or even more successive shoots during a growing season is a common occurrence. When the terminal bud on the stem of an oak resumes growth during the season it is produced, lateral buds on that same segment also usually resume growth and produce side branches. In the willow oak

[1] So-called because they are supposed to develop about August first, which according to the church calendar is "Lammas-day." Actually Lammas shoots usually develop earlier in the summer than this, at least under North American conditions.

(*Quercus phellos*) three or even more prolongations of the same woody axis may take place during a single growing season. Almost always, however, when buds of the current crop on woody stems resume growth there is a short dormant period between the time formation of the bud is completed and the time its active growth is resumed.

Cambial growth as well as the development of the terminal bud meristems is also characterized by a marked seasonal periodicity. Division of the cambium cells in woody stems usually does not start until after expansion of the buds has begun. In many and perhaps all tree species resumption of growth by the stem cambium begins just beneath the enlarging buds and then progresses in a basipetal direction. Cambial activity spreads gradually down the twigs, from the twigs into the branches, from the branches into the trunk and ultimately into the roots (Priestley, 1930). The numerous "waves" of cambial growth which are initiated in this way in the smaller branches apparently become more or less integrated into a single wave as they pass downward into the main axis of the plant. Hence in large trees radial growth may not be resumed in the trunk until several weeks later than in the small branches, and in the roots not until several weeks later than in the trunk. In the roots, however, some cambial growth apparently occurs independently of cambial activity in the stems and trunk.

Suggestions that the downward propagation of a cambial stimulus through the stems of woody plants in the spring may be accounted for in terms of the basipetal movement of hormones have found some experimental support. According to Snow (1935) when aqueous solutions of auxin *a* or indole-3 acetic acid (heteroauxin) were applied to the terminal ends of decapitated sunflower seedlings, division of the cambial cells was initiated. Recent work by Avery, *et al.* (1937) also points to this conclusion. Buds of the horse chestnut and apple in the winter condition failed to give any indication of the presence of active hormones when tested by the oat coleoptile technique. As the terminal buds of both of these species swelled, growth hormone was found to be present in increasing concentrations. The peak concentration was found just prior to the most rapid elongation of the current season's shoots. Hormones were produced both in terminal buds and the current season's shoots. Cambial activity began just below the terminal buds and progressed basipetally into older portions of the stem, following movement of the hormone into those regions. It has also been shown that introduction of a crystal of indole-3 acetic acid into the cambium of willow and other woody species leads to rapid cambial growth in a short zone below the point of insertion of the crystal (Söding, 1936). These observations make it seem

highly probable that vernal resumption of cambial activity occurs under the influence of a hormonal stimulus.

Secondary thickening of the stems of most woody species generally continues until later in the summer than elongation of the current shoots, although usually at a diminishing rate. Cambial activity usually ceases in the young twigs by midsummer, but may continue until late summer or early autumn in the older stems and sometimes until winter in the roots. Cessation of cambial activity in stems during the summer months may often be causally related to the decrease in water content of the stem tissues which usually takes place at that season.

Less is known regarding the seasonal periodicity of the growth of roots than of the aerial organs of plants. The elongation of the roots of woody species apparently begins somewhat earlier in the spring than the elongation or cambial activity of stems. Growth of roots both in length and in diameter continues later into the autumn than the growth of stems. Elongation of roots even through the winter months has been reported by several observers. Harris (1926), for example, records continued growth of apple and filbert roots in Oregon during the winter months. A temporary midsummer slackening in the rate of root elongation has been observed in some species (Stevens, 1931).

Cyclical Periodicity of Vegetative and Reproductive Growth.—The examples of seasonal periodicity which have already been described involve principally variations in growth rates. Growth periodicity is expressed not only in terms of seasonal variations in the quantitative aspects of growth, but also in the production of certain organs at one stage in the life cycle and other organs at another stage. The most prominent periodicity in the qualitative aspects of plant growth is the cyclical production of vegetative and reproductive organs which is exhibited by most species of plants.

The seasonal periodicity of all annual species is very similar, and involves in sequence: (1) seed germination, (2) vegetative development, (3) flowering and fruiting, usually accompanied, at least during the later stages, by slowly diminishing vegetative growth, (4) senescence, and (5) death of all organs except the seeds. All such species are perennial only by their seeds.

The seasonal periodicity of annual species is by no means immutable, however, but can be altered in various ways. Removal of flowers or fruits or both often leads to an acceleration or renewal of vegetative growth (Chap. XXXIV). Similarly change in the length of the photoperiod at the onset of senescence often causes a rejuvenation of vegetative growth (Chap. XXXIII).

The cyclical production of vegetative and reproductive organs is very

similar in all biennial species. Plants of this type develop only vegetatively during their first growing season, producing underground organs which live over winter. In many biennials the leaves are cold resistant and survive the colder months of the year without injury. During their second growing season vegetative development is often renewed, but before long is largely or entirely superseded by reproductive growth. Death of the plant follows closely after the production of seeds and fruits. As with annuals the usual life cycle of biennials can be modified by various circumstances. For example many biennials become annuals when growing in warmer or longer-season climates than those in which they normally behave as biennials.

A greater diversity of cyclical patterns of reproductive and vegetative development is found in perennial species than in those which live for only one or two growing seasons. The following discussion refers primarily to plants of temperate regions. In many woody perennials flowers are produced in the spring before vegetative growth is resumed or concurrently with the early stages in the development of the new leaf-bearing shoots. Examples of species which exhibit this type of periodicity include many fruit trees (peach, cherry, apple, etc.) and many forest tree species (elms, maples, oaks, chestnut, cottonwood, etc.). In some woody species such as the mulberry, in which flowers develop from axillary meristems on the current season's shoot, blooming occurs at the height of the season of vegetative growth. Many woody perennials do not produce flowers until after the season's vegetative growth is nearly or entirely completed. This is true of many species which bear terminal inflorescences at the end of the current season's shoots such as lilac, buckeye, and horse chestnut.

As in woody species, flower production in herbaceous perennials may occur before the vegetative growth of the same growing season, concurrently with the production of stems and leaves, or only towards the end of a period of vegetative development. The first of these types of growth periodicity, which is the least common, is found in certain spring blooming species. The second is also characteristic of many spring blooming herbaceous plants but is by no means confined to such species. The third type of growth periodicity is especially common among summer and fall blooming species, and is characteristic of all species which produce terminal inflorescences on foliage-bearing stems.

Abscission.—Leaf-fall, particularly as it occurs from the stems of deciduous trees and shrubs in the autumn, is a distinctive phenomenon of periodic occurrence in plants. The *abscission* of leaves occurs at their point of attachment to the stem. The phenomenon of leaf abscission is especially characteristic of woody dicots, but also occurs in some herbaceous species such as

coleus, begonia, and fuchsia. In most herbaceous species, however, the leaves are retained even after they die, and only disappear by decay or by mechanical disruption from the plant. In many herbaceous species most or all of the leaves are retained until after the death of the entire shoot system.

Three principal stages can be distinguished in the process of leaf abscission: (1) the formation of an abscission layer, (2) the actual process of abscission or separation of the base of the leaf or petiole from the stem which bears it, and (3) formation of a layer of cells with suberized walls which prevents desiccation of the tissues under the leaf scar (Lee, 1911).

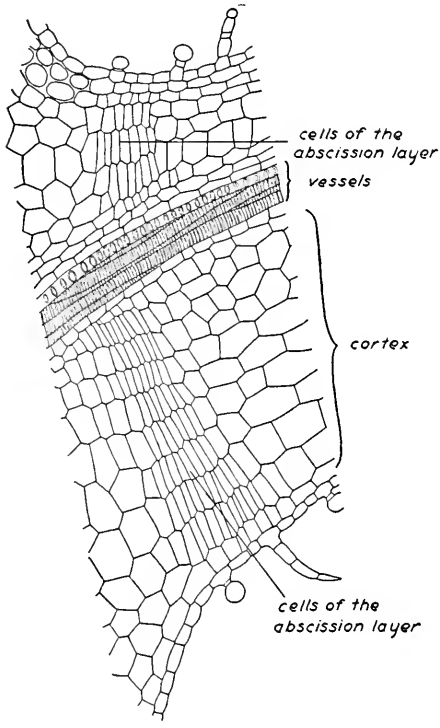

FIG. 143. Abscission layer at the base of the petiole of a leaf of coleus (*Coleus blumei*) as shown in vertical section.

The abscission layer may be differentiated as such weeks or even months before leaf-fall occurs. Typically it develops as a transverse zone of parenchymatous cells at the base of the petiole. This zone of tissue is usually several cell layers in thickness (Fig. 143).

Leaf-fall or abscission proper results from the dissolution of the middle lamella and perhaps also the cellulose wall of these parenchymatous cells. The principal chemical change occurring seems to be the conversion of insoluble protopectin to pectic acid and pectin. After separation of the cells of the abscission layer the petiole remains attached only by the vascular elements. These soon snap off under the pull of gravity or the pressure of the wind, and the leaf drops from the plant. The fractured elements of the vascular bundle usually become plugged with gums or tyloses.

Water loss through the resulting leaf scar is prevented by the formation of a layer of cells the inner walls of which are suberized, and the outer lignified. Subsequently other layers of corky cells develop beneath this layer of cells. Eventually these layers of corky cells which cover a leaf scar coalesce with the corky layers (*periderm*) of the stem proper.

Some of the known causes of the abscission of leaves are: (1) a water deficit in the plant, usually developed as a result of drought conditions, (2) low temperatures, (3) reduced light intensity, (4) change in length of the photoperiod, and (5) destruction or removal of all or most of the leaf blade.

Very little is known of the mechanism whereby any of these conditions induces leaf abscission. The induction of abscission by removal of the leaf blade, an excellent example of a growth correlation, can be easily demonstrated in coleus plants. If the blade of a coleus leaf is pinched off, abscission of the petiole will occur in a day or two (Sampson, 1918). Recent investigations (LaRue, 1935) have shown that if a small portion of lanolin containing auxin is affixed to the stump of a petiole from which the blade has been removed, abscission of that petiole will be greatly delayed as compared with similar petioles which have had only pure lanolin applied at the cut end. This suggests that abscission of leaves is retarded by the migration of a growth-regulating substance from the blade to the base of the petiole. Destruction or removal of the blade eliminates the supply of this substance to the abscission layer, and hence induces abscission.

Leaves, however, are not the only organs or parts of plants which abscise. In compound leaves the individual leaflets usually drop off one by one, leaving the petioles attached to the otherwise defoliated plant. Usually abscission of the petioles follows within a relatively short time. Similarly bud scales, inflorescences, petals, and fruits may be detached from the parent plant by abscission. Segments of the woody stems of some species also abscise. In many species of woody plants (examples are elm, cherry, birch, linden) abscission of the leafy stem tips occurs at the termination of the spring growing period. In such species elongation of the stem continues the next season from the lateral bud just below the point of abscission, such lateral buds functioning essentially as terminal buds. Many species of conifers bear their needle-like leaves in fascicles, each fascicle being attached to a dwarf branch. In the pines and other such species leaves are shed in bundles by the abscission of the dwarf branches rather than by detachment of the individual needles. In certain other woody species (oaks, cottonwood) segments of woody stems of considerable age and diameter are often shed by abscission.

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CHAPTER XXXVII

PLANT MOVEMENTS

The wide variety of movements which occur in the organs of the higher plants usually escape notice because of the slowness with which they take place. By employing the technique of modern motion picture photography, however, it is possible to demonstrate the movements of plant organs in a spectacular manner. If a growing plant is photographed at regular and frequent intervals for a period of several weeks with a motion picture camera and the resulting film run through a projecting machine all the movements which occurred during several weeks of growth take place within a period of a few minutes. In this way the vigor and reality of the autonomous movements of leaves and stems can be demonstrated in a striking manner. The large leaves of tobacco plants, for example, appear to rise and fall almost like the wings of a bird in flight. Likewise the stem tip is seen to participate in more or less regular spiral movements and the several types of movements associated with the expansion of young leaves are vividly portrayed. The movements that occur during the opening of flower and leaf buds can also be demonstrated by this technique. Anyone who has seen such pictures can not fail to be impressed with the many kinds of movements which occur in the aerial organs of plants and with the magnitude of such movements.

Classification of Plant Movements.—Most of the movements exhibited by the organs of the higher plants may be classified as: (1) growth movements, (2) turgor movements, and (3) hydration movements.

1. *Growth Movements.*—Growth movements are changes in the position of organs resulting from an enlargement of cells, or from an increase in the number of cells, or both. Curvatures or other changes in position result from growth movements when the increase in size or number of cells is not uniform at all points in the region undergoing growth. Growth movements are usually divided into three sub-groups: (1) tropic (or tropistic) movements, (2) nastic movements, and (3) nutations.

Tropic movements are those which occur under the influence of environmental factors that act with a greater intensity from one direction than from

another. The direction of the curvatures resulting from such movements often bears a relation to the direction from which the initiating factor acts with greatest intensity. The curvatures of growing stems and roots induced by differences in the intensity or quality of incident light (*phototropic* curvatures) and those resulting under the influence of gravity (*geotropic* curvatures) are the most familiar tropic movements. Similarly changes in the position of organs which are evoked by differences in the water content of the soil, by physical contact, and by specific chemical compounds are known as *hydrotropic, thigmotropic,* and *chemotropic* movements respectively. The movement is considered to be *positive* when the organ bends toward the direction from which the factor is acting and *negative* when it is in the opposite direction.

Nastic movements are those that occur in plant organs when the initiating factor affects all parts of the growing organ uniformly, or when the initiating factor, although acting entirely or principally from one direction, evokes a reaction which occurs in the same manner and in the same direction regardless of the direction from which the factor acts. The growth movements of very young leaves, bud scales, and flower petals are examples of nastic movements. At first the morphologically lower side of these structures grows more rapidly than the upper side so that they are bent upward and enclose the stem tip. This more rapid growth of the lower side is known as *hyponasty* (*hypo*=lower). The opening of the bud is brought about by a more rapid growth of the morphologically upper side of these structures, a phenomenon known as *epinasty* (*epi*=upper).

Although the main stem of most herbaceous plants appears to grow straight upward careful measurements demonstrate that the stem tip actually traces an irregular spiral pathway in space as it elongates. This approximately spiral movement of growing stem tips is known as *nutation*. Nutation results from unequal rates of growth in different vertical segments around the stem axis and often seems to occur independently of environmental factors. The term nutation is sometimes used to include all movements which result from unequal rates of growth. In this book, however, the term will be used only in the more limited sense just specified.

2. *Turgor Movements.*—Movements of plant organs caused by reversible changes in cell volume are known as *turgor movements*. Many of these movements are initiated in compact groups of relatively large, thin-walled cells that constitute the so-called "motor organs" or *pulvini* but they may also occur in any tissue that is largely composed of living, thin-walled cells. Examples of turgor movements associated with pulvini are the spectacular reactions of the sensitive plant (*Mimosa pudica*) to slight shocks or other

"stimuli," and the so-called "sleep" movements of the leaves and leaflets of many other legumes. The opening and closing of stomates and the movements of leaves caused by wilting and recovery illustrate turgor movements that are not associated with pulvini.

3. *Hydration Movements.*—Movements may occur in non-living plant tissues or organs as a result of the hydration or dehydration of the cell walls. These movements are illustrated by the splitting of pods, the opening of capsules, and the rapid movements of mature fern sporangia. Movements of these kinds are not produced by the physiological activities of living cells and will not be considered further in this discussion.

Phototropism.—Phototropic curvatures are familiar to all close observers of plants. They are particularly conspicuous in plants growing in situations in which they are exposed to unequal illumination on opposite sides. Under such circumstances the growing stems usually bend toward the direction of the more intense light and the leaves also become definitely oriented with relation to the light source, regardless of the position of their attachment to the stem. When vines such as the English ivy (*Hedera helix*) are growing on a wall so that light strikes the plants mainly from one direction the leaf blades occupy practically the entire exposed surface with a minimum of overlapping. The leaf blades appear to fit together so exactly that the resulting patterns are known as "leaf mosaics." Similar, though less accurately formed "leaf mosaics" are present in most plants bearing large numbers of leaves. Anyone standing beneath a large maple or oak tree, for example, cannot fail to be impressed by the completeness with which the sky is obscured by the leaf pattern. The leaves of some herbaceous plants (*Lactuca, Silphium,* etc.) are often so oriented that the blade surfaces face the east and west and only the edge of the blade receives the full intensity of the mid-day sun. This orientation is so conspicious that these plants are commonly known as "compass plants." The leaf blades of the turkey oak (*Quercus catesbei*) also have a characteristic vertical orientation under conditions of intense sunlight.

The movements which bring the leaves and stems into the oriented positions just described are caused by differences in the growth rates on the exposed and shaded portions of the stems and petioles. In some species these different rates of growth are sufficiently rapid to become conspicuous. The flowering heads of young sunflowers, for example, face the east in the morning and follow the sun during the day. As soon as growth of the stem ceases the movement likewise ends.

While growing stems and leaves usually react positively to unilateral illumination, roots commonly show no response. However, some roots such as those of white mustard (*Brassica alba*), are negatively phototropic as are the

numerous adventitious roots on the aerial stems of many climbing plants.

Most of our knowledge of the mechanism of phototropic movements has been derived from a study of the behavior of the coleoptile of the oat plant (*Avena sativa*) when subjected to one sided illumination. Because of its reactivity, its structural simplicity, its uniformity of behavior under similar conditions and its general suitability for such work this structure has been widely used in experimental studies of phototropism, geotropism, and some other tropic movements (Chap. XXXII)

It has been known since the time of Darwin that curvature of a coleoptile will occur if only the extreme tip of the coleoptile is exposed to unilateral illumination. The region in which the cell enlargement responsible for this curvature occurs, however, is some distance below the tip (Chap. XXXII). If the tip of the coleoptile is shaded by means of a tin foil cap and the entire coleoptile illuminated unilaterally little or no curvature results. Likewise, decapitated coleoptiles react feebly to one sided illumination, but if coleoptile tips which have been subjected to one sided illumination are placed upon unilluminated coleoptile stumps, marked phototropic curvature of the stump results. Experiments like these indicate clearly that the tip of the coleoptile profoundly influences the enlargement phase of growth in cells below the tip of the coleoptile.

The positive phototropic curvature of oat coleoptiles is caused principally by greater elongation of the cells on the shaded side of the coleoptile than of the cells on the illuminated side. Since cell elongation is known to be influenced by the quantity of auxin present (Chap. XXXII) it is logical to seek an explanation of such phototropic reactions by studying the effect of light upon the distribution of auxin in the coleoptiles. Extensive investigations have shown that, in oat coleoptiles at least, unilateral exposure to light increases the quantity of the auxin reaching the shaded side of the coleoptile from the tip and decreases the quantity on the illuminated side. The phototropic curvature of oat coleoptiles and presumably of many other plant structures apparently results from the presence of unequal quantities of auxin on the two sides of the coleoptile. Some of the evidence upon which these conclusions are based will be reviewed briefly.

Went (1928) removed the tip of a coleoptile that had been exposed unilaterally to light of suitable intensity and placed it upon two small blocks of agar, separated from each other by a thin metal plate, in such a way that the auxin from the shaded and illuminated sides diffused into different agar blocks (Fig. 144). The blocks were then tested for auxin content by the oat coleoptile technique. The resulting curvatures indicated that more auxin diffused out of the shaded half of the coleoptile tip than out of the illuminated

half. Furthermore, the quantity of auxin from the shaded half was appreciably more than the amount from half of an unilluminated tip. Went concluded therefore, that one-sided exposure to light had caused some of the auxin to migrate from the illuminated to the shaded side of the coleoptile.

Boysen-Jensen (1928) came to a similar conclusion from a different kind of an experiment. The tip of a coleoptile was split longitudinally, a thin glass plate was inserted between the split halves and the coleoptile was exposed to unilateral illumination of suitable intensity. When the glass plate was parallel to the light beam, normal phototropic curvatures occurred, but when the glass plate was at right angles to the direction of the light very little curvature resulted. When at right angles to the light beam the glass plate apparently prevented the lateral migration of auxin from the lighted to the shaded side of the coleoptile so that the quantity of auxin was approximately the same on both sides. When the glass plate was parallel to the light beam, however, movement of auxin from the lighted side was not prevented. This experiment also indicates that under conditions of one sided illumination, auxin migrates from the illuminated to the shaded side of the oat coleoptile and that positive phototropic curvature is correlated with the greater auxin content of the cells on the darker side of the coleoptile.

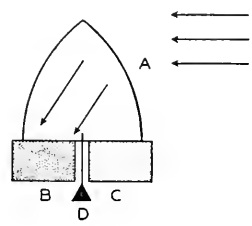

FIG. 144. Diagram to show method of demonstrating results of unilateral illumination upon auxin distribution in coleoptile tips. (*A*) coleoptile tip, (*B*) and (*C*) agar blocks, (*D*) metal plate. Horizontal arrows indicate direction of illumination. Auxin is displaced towards side of coleoptile away from light.

Burkholder and Johnston (1937) have shown that light of high intensity may cause a destruction or inactivation of auxin in plant tissues. It is possible therefore that under certain conditions phototropic curvatures of oat coleoptiles may be caused in part by an inactivation of auxin upon the illuminated side and in part by a migration of auxin from the lighted to the shaded side of the organ.

All wave lengths of the visible spectrum are not equally effective in inducing phototropic curvatures. The shorter wave lengths are most effective and the longer wave lengths at the red end of the spectrum evoke practically no phototropic reaction. According to Johnston (1934) the most effective wave lengths lie in the range from 440 $m\mu$ to 480 $m\mu$ (Fig. 145).

In the early years of the present century Blaauw demonstrated that a certain minimum quantity of light was essential for a perceptible phototropic curvature of an oat coleoptile. Since quantity is a product of intensity and

duration, low intensities are effective only upon long exposures but with high intensities extremely short exposures are sufficient. With a light intensity of only 0.00017 meter candles an exposure of 43 hours was required to induce

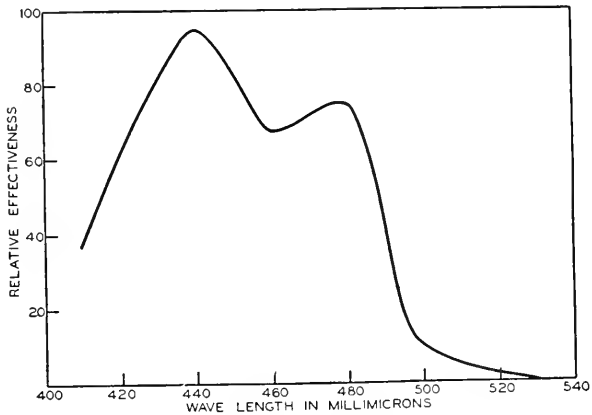

FIG. 145. Relation between wave lengths of light and phototropic curvature of oat coleoptiles. Data of Johnston (1934).

phototropic curvature but with a light intensity of 26,520 meter candles an exposure of only 0.001 second resulted in phototropic curvature.

The minimum quantity of light required for phototropic movement, the so-called "threshold value," varies greatly with the portion of the coleoptile tip that is illuminated. The terminal 0.5 mm. of the coleoptile, for example, is nearly 1600 times as sensitive to illumination as a zone only 1.5-2.0 mm. below the tip (Lange, 1927).

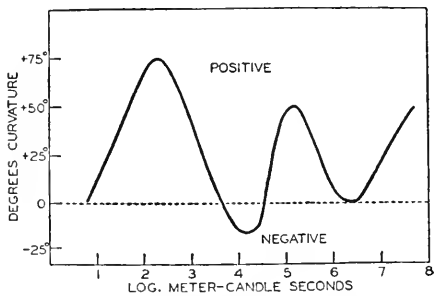

FIG. 146. Relation between phototropic curvature and quantity of light received unilaterally. Data of du Buy and Nuernbergk (1934).

Experimental results of a number of investigators have shown that the degree of phototropic curvature is controlled by the quantity of unilateral light. The curvature is not directly proportional to the amount of light but varies periodically as the quantity of light is increased (Fig. 146).

As the data in Fig. 146 indicate, once the threshold value is passed the coleoptiles curve toward the source of light and over a certain relatively

low range of light intensities the degree of their curvature is proportional to the quantity of illumination. With further increase in the quantity of light, however, this relationship changes and the degree of phototropic curvature decreases until negative curvatures are evoked. Still greater quantities of light induce a second series of positive curvatures. On increasing the quantity of light still more the coleoptiles react with decreasing curvatures until a point is reached at which the light induces no visible end-reaction. Further increases in light quantity beyond this point evoke a third set of positive curvatures.

A correlation exists between these variations in the magnitude of phototropic curvatures and the quantity of auxin diffusing from the illuminated and shaded sides of coleoptile tips (Table 60).

TABLE 60—THE RELATION BETWEEN THE AUXIN CONTENT OF COLEOPTILE TIPS SUBJECTED TO UNILATERAL ILLUMINATION OF DIFFERENT INTENSITIES AND THE DEGREES OF PHOTOTROPIC CURVATURES (FROM DATA COMPILED BY WENT AND THIMANN, 1937)

Amount of light, meter candle seconds	Type of curvature	Extent of curvature	Auxin distribution in per cent	
			Lighted side	Shaded side
0	Control	0°	49.9	50.1
20	First positive	+ (10°)	41	59
100	First positive	++	26	74
500	First positive	++	36	64
1,000	First positive	++(48°)	32	68
10,000	Indifferent	About 0°	49	51
?	First negative	.....	58	42

Auxin concentration is not the only factor involved in phototropic movements. The quantity of growth hormone can only control the extent of cell elongation when it acts as a limiting factor. We have seen (Chap. XXXII) that auxins are produced in the coleoptile tip and move basipetally into other parts of the structure. It is not probable, therefore, that curvatures of the upper portions of the coleoptile are due to differences in auxin content for the hormones are present in excess in these regions. Photographic measurements have shown that in the upper zones of the coleoptile, curvatures are not produced by increases in the rate of elongation of cells on the shaded side but by a decrease in the amount of cell elongation on the illuminated side. Likewise uniformly illuminated coleoptiles grow more slowly than coleoptiles kept in the dark. Numerous other experiments also indicate that

light has a direct effect upon growth independent of its influence upon the auxin distributing mechanism of the plant (van Overbeek, 1936a, 1936b).

The relative importance of this "light-growth" reaction in phototropic movements is somewhat uncertain. It seems clear that phototropic curvatures are not simple light-growth reactions as was widely believed at one time, for it has been demonstrated that the light-growth effect is inadequate to account for the differences in growth rates that are responsible for most phototropic curvatures. The effect of unilateral illumination upon the distribution of auxin appears to be the primary cause of phototropic curvatures and the direct influence of light upon the growth of cells seems to be of secondary importance.

Geotropism.—If a potted plant be placed in a horizontal position for a few days the stems no longer lie prostrate but begin to turn upward away from

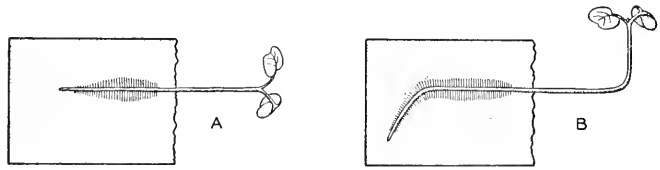

FIG. 147. Diagram illustrating geotropic curvature of root and hypocotyl of a mustard plant. (*A*) plant just after having been placed horizontally, (*B*) one day later.

the direction of gravitational attraction. This change in position first appears in the region of elongation just back of the stem tip and with time may extend backward toward the older portions of the stem. If the primary root tips of the plant are examined they will also be found to have altered their position, but in exactly the opposite direction, by growing downward toward the center of the earth. The behavior of roots can be more easily observed in germinating seeds and as in stems the change in position first appears in the region of elongation just back of the root tip (Fig. 147).

If horizontally placed potted plants are rotated slowly about the stem as an axis, so that every vertical segment of the stem becomes successively the upper and then the lower side, no geotropic curvatures appear. No segment of the stem or root remains long enough in any one position for growth curvatures to occur. Similarly the roots fail to curve when germinating seeds are fastened to the rim of a wheel which is rotated rapidly in a horizontal plane. Growth is more strongly influenced by the centrifugal force generated by the rapid rotation than by the force of gravity. The roots react positively

and grow toward the direction of the force (*i.e.* outward) while the stems grow away from the direction of the force (*i.e.* inward toward the hub of the wheel). If the rotation of the wheel is stopped or slowed down to a point where the centrifugal force is less than the pull of gravity, the usual geotropic curvatures of both root and stem soon appear.

Decapitated primary roots usually fail to exhibit geotropic curvatures when placed in a horizontal position even though the amputation of the root tip does not prevent the enlargement of cells below the tip. If tips from vertically oriented roots are placed upon such decapitated horizontal roots positive geotropic curvatures appear. Such experiments indicate that the tip of the root exerts a predominating influence upon its geotropic movements, a relation comparable to that of the coleoptile tip in phototropic reactions of the coleoptile.

In stems, however, geotropic reactions are not prevented by amputation of the stem tip. In general the stem tip is more "sensitive" to the force of gravity than zones at some distance below the tip but this "sensitivity" to gravity is usually present throughout the growing region. In many grasses geotropic reactions occur in mature nodes independently of the stem tip.

The quantitative measurements of auxins made possible by the oat coleoptile technique have been utilized successfully in studying the rôle of auxins in geotropic curvatures. Coleoptiles which are slowly rotated around their own vertical axis while in a horizontal position do not differ from vertically oriented coleoptiles in their rate of growth. Tips of the horizontally placed coleoptiles produce the same amount of auxin as vertically oriented tips. The negative (upward) curvatures of the coleoptiles induced by gravity are not the result, therefore, of any total increase in the amount of hormones produced on the lower side of the coleoptile tip. When the amount of auxin diffusing out of the upper and lower halves of horizontally placed coleoptile tips is determined by the agar block method it is found that more than half of the auxin diffuses out of the lower half of the tip (Navez and Robinson, 1932). Apparently, gravity, like light, influences the distribution of the auxin in the coleoptile and the upward curvatures obtained as a consequence of the force of gravity are due to the greater concentrations of the hormone on the lower side of a horizontally placed coleoptile. Unlike the effect of light, however, the influence of gravity upon the distribution of the auxin within the coleoptile does not persist for very long after the organ has been returned to its original position. The effect of gravity upon curvature is lost within forty minutes after the removal of the "stimulus" but the effect of light upon the distribution of auxin in the cells of the coleoptile may persist as long as six hours after removal of the light.

The same concentration of auxin which favors the elongation of stems and coleoptiles retards the elongation of roots (Chap. XXXII). This fact suggests that the positive (downward) geotropic reaction of root tips may be caused by the same mechanism which invokes the negative (upward) curvature of stems or coleoptiles. A greater concentration of auxin in the lower half of horizontally placed roots would check elongation rather than increase it and the growth of the upper side of the root would exceed that of the lower, resulting in its downward curvature. Experimental tests have confirmed this explanation. The lower halves of tips of horizontal roots have been found to contain higher concentrations of auxins than the upper halves (Hawker, 1932). Since the growth rate of primary roots which are slowly rotated about their own axis in a horizontal plane does not exceed that of vertical roots (*i.e.* auxin concentrations are equal in the root tips in both positions) the greater quantity of auxin in the lower half of the tips of horizontal primary roots indicates that the auxin migrates to this region under the action of gravity. The downward curvature of root tips is therefore found to be correlated with the auxin concentration.

Until the comparatively recent work upon auxins geotropic curvatures were commonly explained by the *statolith theory*. According to this theory the geotropic curvatures of stems and roots were caused by changes in position of mobile solid bodies, such as starch grains, in certain "sensory cells" under the action of gravity. The weight of these bodies upon the cytoplasm of the sensory cells was believed to initiate a chain of events which ultimately resulted in geotropic curvature. The statolith theory is now chiefly of historical interest since it is possible to demonstrate experimentally a quantitative relationship between auxins and geotropic curvatures in a number of different plants.

Thigmotropism.—The growth movements made by plants as a consequence of contact with solid objects are known as thigmotropic reactions. These movements are best illustrated in the growth of tendrils, though they are also exhibited by petioles, stems and other organs of some plants. Tendrils are slender cylindrical organs that structurally represent modified stems, leaves or leaflets. Some common tendril-bearing species of plants are the grape vine, greenbriers (*Smilax*), sweet pea and wild cucumber (*Sicyos*). As a result of unequal rates of growth the tips of young tendrils exhibit the phenomenon of nutation (see later) and make slow circular movements in space during their elongation. As soon as a tendril comes in contact with a solid object, rapid growth reactions are initiated. The cells on the side which makes contact with the solid object shorten somewhat and the cells on the opposite side quickly elongate with the result that the tendril is bent around the sup-

port. This movement usually occurs within a few minutes, and in the tendrils of some species may take place in less than a minute. The speed of the reaction is such as to suggest turgor changes in the cells rather than growth. However, since the resulting changes in cell size are irreversible the movement is properly classed as a tropism. Once the tendril becomes attached to some object further growth in length ceases. As a result of inequalities in growth in the basal region the tendril becomes spirally coiled so that it resembles a coil spring. Secondary wall formation then follows, transforming the delicate thin-walled tendril into a firm supporting organ.

A young tendril reacts readily to contacts with very light solids provided the solid surface is not perfectly smooth and that contact with the tendril is made at more than one place. No growth movements occur in tendrils as a result of contacts with liquids or perfectly smooth solids. If a very light thread weighing a small fraction of a milligram be moved along the surface of the tendril curvatures will result, but drops of mercury many thousand times heavier, or rain drops bring about no reaction. The mechanism of thigmotropic movements cannot be satisfactorily explained at the present time.

Hydrotropism.—The roots of plants do not grow into soils in which the water content is at or below the wilting percentage but usually do grow into soils of higher water content. When the soil in small pots containing growing plants is watered by means of porous clay irrigators the roots of plants are often matted heavily around the surface of the irrigators and are more sparsely distributed in the surrounding soil (Hendrickson and Veihmeyer, 1931). Such observations have often been cited as examples of positive hydrotropism in roots.

The work of Loomis and Ewan (1936) suggests, however, that curvatures of growing root tips toward regions of higher water content are not as common as was once supposed. These investigators filled shallow boxes half full of moist soil, placed the seeds to be observed upon the surface of the moist soil, and then filled the box with dry soil. The moist soil was near its field capacity and the dry soil had a moisture content well below the wilting percentage (Chap. XVI). The soil was held firmly in position with paraffined paper and the box placed in a moist chamber in such a way that the boundary between the moist and dry soil made an angle of 45° to the vertical and so that the dry soil was on the lower side of this boundary. These conditions made it possible to determine the relative influence of soil moisture and gravity upon the direction of root growth. A positive hydrotropic reaction would result in a bending of the primary roots toward the moist soil. Examination of the root growth of thousands of seedlings representing 26 species

revealed that few species exhibited positive hydrotropism. In most species the roots that started to grow downward as a consequence of gravitational attraction soon ceased to grow because of insufficient water. Roots that happened to be produced in the moist soil grew normally. Positive hydrotropism was found to be present in a few species of the *Cucurbitaceae* and *Leguminosae* since the roots of seedlings of these species curved away from the dry soil toward the moist soil and grew along the boundary between the moist and dry soil. The experiments demonstrate that hydrotropic curvatures do occur in the soil grown roots of some species but also indicate that such curvatures are probably not a common phenomenon under field conditions.

Hydrotropic curvatures are caused by differences in the rate of enlargement of the cells on the opposite sides of the root but no satisfactory explanation of the cause of this unequal growth has been suggested.

Traumatotropism.—Injury to the tissues of plants, especially to stems and roots, often results in curvatures caused by unequal growth. Such curvatures are examples of traumatotropic reactions. Transverse incisions in oat coleoptiles commonly result in positive curvatures but decapitated coleoptiles usually fail to react to injury inflicted near the upper end. Incisions made near the base of decapitated coleoptiles may induce positive curvatures, however. From the evidence available it seems probable that injuries influence either the distribution of auxin in the tissue (Keeble and Nelson, 1935) or interfere with the transport of foods or other substances into the growing regions from other parts of the plant, but no more detailed analysis of the mechanism of these reactions is possible in terms of currently available information.

Nastic Movements.—The essential distinction between nastic and tropic movements has already been described. With few exceptions, nastic movements are evoked by environmental factors such as temperature or diffuse light which influence the organ with equal intensity from all directions, while tropic movements are induced by factors which act with greater intensity from one direction than others. Equal illumination of all parts of a plant organ, for example, may result in *photonastic* movement, causing the organ to assume a different position in the light than in the dark. *Phototropic* movement of a plant organ occurs, however, only when the organ is illuminated unequally from different directions. Furthermore, nastic movements are chiefly restricted to organs such as leaves and petals, the structure of which largely or entirely prevents their movement except in certain directions.

The leaves of a number of different species of plants undergo marked changes in position during the day and night. In some species the leaves droop at night and become oriented more or less horizontally during the day.

The common jewel-weed (*Impatiens*) and some other members of this family react in this way. The leaves of other species, notably the pigweeds (*Amaranthus*), move upward into a more nearly vertical position at night from the approximately horizontal position occupied during the day. Both of these kinds of movements are caused by differences in the rate of growth on the two sides of the leaf. The downward movement of leaves in the dark is the result of a more rapid growth upon the upper than upon the lower surface of the petiole while in those species in which the leaves move upward in the dark the growth rate is more rapid upon the lower side of the petiole. The movements just described are growth movements and cease entirely when the leaf reaches its full size.

Many of the movements of leaves and leaflets, however, are turgor movements rather than growth movements. Such movements are discussed later in the chapter.

Some flowers also exhibit such photonastic movements. *Oxalis* flowers, for instance, close at night while those of the evening primrose (*Oenothera*) open during the evening and night and close early on the following day as a consequence of photonastic reactions (Goldsmith and Hafenrichter, 1932).

Temperature changes may likewise bring about nastic movements of the flower petals of some species. In *Crocus* flowers, for example, an increase in temperature produces a more rapid growth of the inner surface of the petals than on the outer side resulting in the partial or complete opening of the flower. A decrease in temperature has the opposite effect, increasing the growth rate on the lower side more than that of the upper surface.

Nastic movements are growth movements and might, therefore, be expected to have some relation to the distribution of auxins in the tissues affected. Zimmerman and Wilcoxon (1935) were able to produce epinasty in the leaves of several species of plants by the application of different growth promoting substances. Avery (1935) demonstrated that typical nastic responses can be produced by the application of small amounts of auxins to the base of the petiole of tobacco leaves. Although the mechanism of the action is not clear it seems probable that nastic movements like many tropic movements are causally related to the distribution of auxins in the tissues concerned.

Nutation.—All growing stem tips describe an irregular spiral path in space as they elongate. This phenomenon, as previously mentioned, is known as *nutation* and it appears to be caused, in most plants at least, by internal factors that affect the growth rate of vertical segments of the stem in the region of elongation.

The most striking examples of nutation are found among twining plants. The nutation of the stem tips of such species is at least partly a consequence

of the influence of gravity. The stem tips of twiners are usually long and slender and devoid of leaves. Their mechanical tissues are not well developed so that the tip of the stem usually droops over into a more or less horizontal

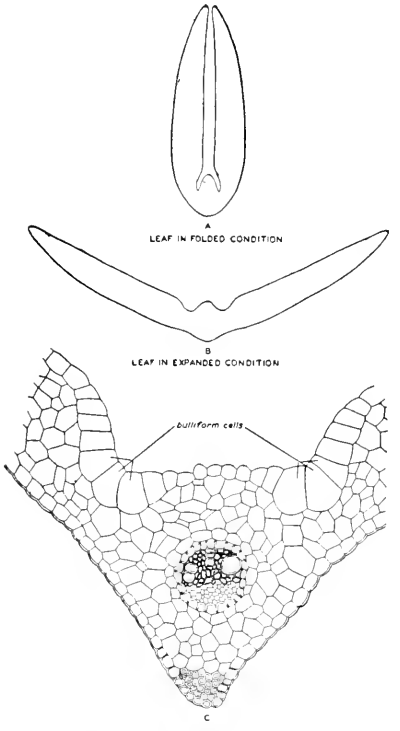

FIG. 148. (*A*) outline drawing of blue grass (*Poa pratensis*) leaf in folded condition, (*B*) in an expanded condition, (*C*) detailed drawing of mid-portion of the leaf showing the "bulliform cells," changes in the turgor of which determine the folding and opening of the leaf.

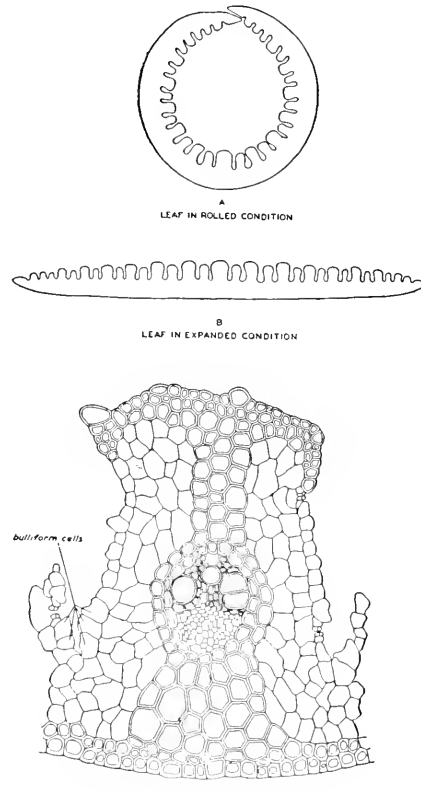

FIG. 149. (*A*) outline drawing of sand reed (*Ammophila*) leaf in rolled condition, (*B*) in an expanded condition, (*C*) detailed drawing of mid-portion of the leaf showing a few of the "bulliform cells," changes in the turgor of which are responsible for the rolling and opening of the leaf.

position. More rapid growth on the lower and outermost sides of the stem results in the upward swinging movements of the tip. This growth is associated with a twisting of the stem which follows as a result of the unequal growth rates so that different segments of the tip become successively placed

on the lower side of the stem. It has been pointed out earlier that growth substances are known to accumulate in the lower half of horizontally placed stem tips. It seems probable that similar accumulations of auxin may influence the nutational movements of the stem tips of twining plants.

Turgor Movements.—In contrast to the growth movements which always involve a permanent increase in the size or number of cells in the tissues concerned there are many movements which result from reversible changes in cell size. These are caused by variations, sometimes very rapid, of the turgor pressure in the cells concerned. Because of their dependence upon changes in turgor pressure, movements of this kind are known as *turgor movements.*

Turgor movements are responsible for the folding or rolling of the leaves of many grasses during wilting, for the so-called "sleep movements" that occur at night in the leaves of a number of species and for the sudden and spectacular movements that occur in the "sensitive plant" (*Mimosa pudica*) under the influence of various "stimuli."

Many turgor movements are caused by variations in the turgor of specialized cells or organs. The folding and rolling movements of blue grass leaves, for example, are produced by turgor changes in large, thin-walled cells that are located on the upper leaf surface at the base of two grooves that run parallel with the principal veins (Fig. 148). When turgor is high the distention of these cells holds the leaf blade expanded and relatively flat but when the turgor pressure of these cells decreases the pressure of the cells on the opposite side of the leaf forces the leaf blade to fold.

In the beach grass (*Ammophila*) such cells occur at the base of a number of grooves in the upper surface of the leaf, and upon the loss of turgor of these cells the leaf rolls up (Fig. 149).

In some species of plants movements result from turgor changes in the cells of *pulvini.* These structures are found in many species of the *Leguminoseae.* Pulvini are commonly located at the base of the petiole and at the point of attachment of the leaf blade to the petiole. Externally they appear as short, more or less swollen portions of the petiole. When pulvini are present in compound leaves there is usually one at the point of attachment of each leaflet to the petiole as well as one at the base of the petiole. A pulvinus is composed of a compact mass of large, thin-walled cells which surround a central vascular strand (Fig. 150).

When all of the cells in a pulvinus are distended by their turgor pressure the leaf is firmly supported. Movements result from sudden changes in the turgor of the cells in one portion of the pulvinus while the turgor of cells on the opposite side is maintained or even increased. The unequal pressures thus arising on the two sides of the pulvinus cause the petiole to

move toward the side having the reduced pressure. As the flaccid cells of the pulvinus regain their turgor the petiole is pushed slowly back into the position occupied before the movement occurred. Frequently the loss of

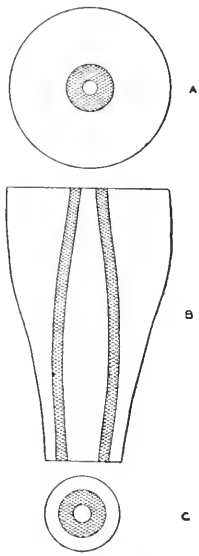

FIG. 150. Diagrams showing distribution of the vascular tissue (shaded) in (*A*) cross section of a pulvinus, (*B*) the transition zone between a pulvinus and a petiole (longitudinal section) and (*C*) cross section of a petiole.

FIG. 151. A sensitive plant (*Mimosa pudica*). Upper: leaves in expanded condition; lower: leaves in a collapsed condition as a result of turgor movements. (From Stiles *Introduction to the Principles of Plant Physiology,* Methuen & Company, Ltd.)

turgor occurs very rapidly. In the sensitive plant detectable turgor movements have been reported to occur within 0.075 second after "stimulation," and the response may be complete in little more than a second. Recovery of turgor commonly occurs in from 8 to 20 minutes. The speed of the reaction and the time of recovery vary greatly with the intensity of the causal

factor, the resulting movement being more rapid and recovery slower when the initiating factor is intense than when it is weak. In many species, however, pulvinal movements are too slow to be noticed without measurement.

The mechanism causing the sudden changes in turgor of the cells in one part of the pulvinus is not clearly understood. Water moves out of the cells into the adjacent intercellular spaces and some probably enters other nearby cells of the petiole or stem. This outward movement of water from the cells into the intercellular spaces appears to be accompanied by an increase in the permeability of their cytoplasmic membranes and also by a decrease in the osmotically active contents of these cells (Blackman and Paine, 1918). All of these changes are reversible since turgor may be regained by the flaccid cells of the pulvinus within a short period of time.

Turgor movements may be initiated in many different ways. In the sensitive plant (Fig. 151) movements result from physical contact, injury, exposure to various gases, electrical shock, jarring, insufficient water supply, the change from light to darkness and *vice versa* and from other factors as well.

The sensitive plant also exhibits a difference in reactivity to the various wave lengths of light. Turgor movements result when darkened plants are illuminated by wave lengths of light of suitable intensity in the blue, the long ultraviolet and the long red. No movements occur from exposure to wave lengths in the orange, yellow green, or infrared (Burkholder and Pratt, 1936).

The environmental factors initiating the movements may be received by organs at considerable distance from the pulvinus in which the turgor changes causing the movement actually take place. If a terminal leaflet of one leaf of a large sensitive plant is burned by a flame all of the leaves on the entire plant may react with vigorous turgor movements. The conspicuous turgor movements that may easily be evoked in this plant have interested many students and the phenomenon has been exhaustively studied, especially with reference to the mechanism of "stimulus" transmission (Houwink, 1935). When the "stimulus" is mild (cool drops of water applied to the leaflets) it appears to be transmitted only through living cells and the rate of transmission is controlled by the temperature. When a leaflet is injured by burning or cutting, substances appear to be produced at the point of injury, and the transmission of these compounds through the non-living vessels of the vascular system apparently causes the reaction of pulvini located at some distance from the point at which the injury occurred. Whatever may be the mechanism by which reactions are induced at some distance from the point at which the initiating factor acted, the effect is the same: a rapid loss of turgor in the cells

on one side of the pulvinus coupled with a maintenance or even an increase in turgor in the cells on the opposite side of the organ.

Discussion Questions

1. Roots of poplar trees and many other woody species often cause trouble by plugging up sewer lines and drains with masses of profusely branched roots. This has been cited as evidence of positive hydrotropism. Can you see any objections to such an explanation?
2. What explanations can you suggest to account for the failure of most roots to exhibit negative phototropism?
3. The influence of relative moisture content upon the direction of root growth is sometimes demonstrated by planting seeds in a tray with a cheesecloth bottom. The tray is then filled with wet moss and suspended in the air at an angle of 45° to the vertical. When the air around the tray is saturated the roots grow straight down, but when the air is appreciably below saturation the roots bend and grow parallel to the bottom of the tray. Can the results of this experiment be interpreted in any other way than as a reaction to moisture?
4. Leaf mosaics are commonly interpreted as a direct result of light of different intensities. Are any other explanations possible?
5. The very rapid movements of the leaf blades of the Venus Fly Trap are attributed to growth while the rapid movements of the leaves of the Sensitive Plant are classed as turgor movements. How can growth movements be distinguished from turgor movements?

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AUTHOR INDEX

(Boldface type indicates bibliographic reference; asterisk indicates tabulated data, curves, or illustrations from the work of the author cited.)

SUBJECT INDEX