PRINCIPLES OF PLANT PHYSIOLOGY

ORAN RABER

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THE MACMILLAN COMPANY
NEW YORK · BOSTON · CHICAGO · DALLAS
ATLANTA · SAN FRANCISCO

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THE MACMILLAN COMPANY
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TORONTO

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BY

ORAN RABER

Revised Edition

NEW YORK
THE MACMILLAN COMPANY
1938

· PRINTED IN THE UNITED STATES OF AMERICA ·

Set up and electrotyped. Published May, 1933. Reprinted May, 1936 ; May, 1937; May, 1938.

Published June, 1928.

To all those who are interested in the principles of plant physiology and to the many friends who have made this book possible, it is respectfully dedicated.

The rules of the game are what we call the laws of nature.—*Huxley.*

PREFACE TO THE SECOND EDITION

During the past five years since the first edition of this book appeared, it has met with more favor in the eyes of teachers and students of the subject than the author (or the publishers) had anticipated even in the most sanguine moments. But during those five years much phytophysiological water has passed under the botanical bridge. Once again has come the task of deciding what matter shall be included and what shall not. There is also the question of maintaining the book within a reasonable size. No self-respecting student can be expected to carry an "elementary" book of more than 500 pages. Most gospels are small; but revisions have a tendency to get bigger and (let us hope) better.

An elementary text should be "up to date" and yet not contain many disputable data. However, if one included in a textbook only those *facts* to which *every* plant physiologist subscribed wholeheartedly *all* of the time, the author would finish with a mere pamphlet. A happy medium must be sought. Whether the teacher-professor who reads this will think this golden mean has here been attained, will depend upon his personal predilections and the number of fields in which he is *not* a specialist.

So in this, as in the previous edition, there has been an attempt to hold as much as possible to the essentials of a general, elementary course, bearing in mind that this elementary course may be preparing the student for courses in horticulture, agronomy, or forestry; for more advanced courses in plant physiology; or for a more appreciative life in the nonprofessional world.

This edition, therefore, presents no radical changes from the first edition. Many of the sections have been enlarged, and in some chapters new sections have been added, where further expansion seemed warranted by advances in those particular fields. New references, of course, have been included; the selections are varied and should appeal to many types of student-readers. The order of presentation is essentially unchanged. There are some who think that, because the plant absorbs water before it begins making carbohydrates, absorption should be treated before photosynthesis, or that, because "in actual life probably all respiration has an anaërobic start," anaërobic respiration should be taught first; but there is an old rule of pedagogy which states that it is better to proceed "from the known to the unknown" than in the opposite direction. This has been found to hold true in both the college and the kindergarten and there seems no excellent reason to depart now from this accepted principle.

The writer again has to thank his colleagues for their kindness in pointing out errors in the previous edition, in making suggestions for the book's improvement, and (last but by no means least) in showing their appreciation by using it in their classes. Special thanks must be given to the men who obligingly acceded to the request for their photographs, and to Dr. H. S. Conard for reading the proof and for his many helpful offices.

ORAN RABER

Biological Abstracts, Branch Office,
U.S.D.A., Washington, D. C.,
April, 1933.

PREFACE TO THE FIRST EDITION

The writer of a new book in an old field must give a reason for his work. As a teacher of plant physiology I have felt a need for a teachable text for elementary students. We have some very fine reference books for collateral reading, but a modern text written like a text in other fields is at present not to be found. This book, which has grown out of a series of lectures given at the University of Wisconsin and later at the University of Arizona, is planned to meet the need for a new presentation.

Let me emphasize that this is a book for *students* and not for *teachers*, a textbook and not an exhaustive treatise. I have tried to make a connected story which the student can follow, and although my account may be unliterary I hope that it is readable and comprehensible.

Plant physiology is largely a combination of elementary botany, physics, and chemistry; and I have therefore assumed that the student has an elementary knowledge of these contributing sciences. Often a problem of plant physiology can be clarified by material from animal or general physiology, and I have not attempted to draw the line too sharply between these various phases of physiology. Chapters XII–XV, which are chiefly chemical in nature, are really phases of phytophysiological chemistry but are needed by the student if he is to appreciate the work of the plant as a living factory.

I have aimed to make the drawings and illustrations as helpful as possible. The photographs of botanists were very kindly supplied to me in response to a request which I sent to a number of men, and are of living workers in the field. The student too often gains the impression that he is studying a dead science, which has been developed chiefly by men no longer alive.

The questions are intended (1) to call attention to matters mentioned in the text which the student might otherwise neglect, (2) to stimulate thought and reflection, and (3) to encourage the student to go to other sources of information. Most of the questions belong in categories (2) and (3). They are not answered in the text at all or only incompletely. They are meant as outside reading assignments and not as review questions. The student should early get the idea that plant physiology is a *subject* and not a *book*.

The references at the ends of the chapters are intended to supplement the text, and are not merely lists of articles cited in it. Some students enjoy going a little deeper into a subject than their fellows and for them these lists of references are appended. The readings suggested are all in English as I have seldon found that beginning students in America or England are able to handle a foreign language profitably. References in a foreign tongue seem to repel them rather than to attract. To gain the students' interest, I have not hesitated to include in the list popular summaries of a subject as well as the reviews found in the *Botanical Gazette* and *Science Progress.* Completer bibliographies can always be obtained from the articles cited. All that a real student needs is to be placed at one end of the bibliographic trail.

The use of the term "survival value" may need some explanation. An effort has been made to get away from "adaptation" which is anathema to most physiologists. We have all long recognized it as a shorthand expression for which something better was needed. I have hit upon the phrase "survival value" which expresses the idea of the importance of a structure in evolution without at the same time implying something further which we do not intend.

Agronomists may wish there was more mention of field crops, horticulturists may lament the lack of horticultural illustrations, and general physiologists may possibly decry the lack of references to work done on the simpler plants and animals, but a book in general plant physiology must be an introduction to all of its branches; specialization can then follow later.

No one writes a book without being indebted to all who have gone before, and in writing a scientific textbook this is especially true. To my colleagues who have helped with their photographs, I am very grateful; and to Drs. Henry Hooker, O. L. Inman, Antoine Kozlowsky, C. J. Lyon, Wm. Seifriz, and R. H. True who have read parts or all of the manuscript I owe my especial thanks. This is scant gratitude for so valuable a service.

ORAN RABER

Biological Abstracts,
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PRINCIPLES OF PLANT PHYSIOLOGY

CHAPTER I

PLACE OF PLANT PHYSIOLOGY; CLASSIFICATION OF THE BOTANICAL SCIENCES

> The world is so full of a number of things,
> I'm sure we should all be as happy as kings.
> —STEVENSON.

The Sciences.—Owing to the multiplicity of facts in the world, man has found it convenient to classify and arrange those he knows into certain groups. Thus have arisen the various fields of knowledge such as history, botany, music, etc. In common speech those organized bodies of fact like astronomy and geology which deal with material things in the realm of nature are called *sciences*, while those such as music, history, and logic which deal with ideas, emotions, and feelings are not ordinarily so called. The ancients, however, called all these organized groups of facts "sciences" and, for our purpose in this chapter, we shall so consider them.

There are then three great associations of sciences,—the physical, the biological, and the psychological. The physical sciences are concerned with nonliving matter and include physics, chemistry, meteorology, etc. The biological sciences include botany and zoölogy,—the sciences of living things; while the psychological sciences include psychology, and, in a philosophical sense, logic and mathematics.

The most casual observer, however, will not fail to see that even the best classification cannot be "cut and dried" and cannot separate the fields of knowledge into "water-tight compartments." If we place physics at the red end of a knowledge spectrum, biology in the middle in the green portion, and psychology in the violet end, we shall thus have a series of fields of knowledge which, like the wave lengths we have used to represent them, really merge into one another. Physics is linked to biology by biophysics, and biology is connected by history and political science with psychology. But physics is also related to psychology through logic and mathematics, so that instead of representing the fields of knowledge by a spectrum, it would be better to use a color triangle as suggested by Newton (Fig. 1). In such a triangle we can place every known field of knowledge. Music stands between physics and psychology since this latter includes the group of studies which deals with ideas, emotions, and feelings. Geology is between physics and biology; while astronomy is not far from geology, physics, and mathematics. In the center, occupying the place of white light, which is the synthesis of all, stand metaphysics and philosophy, which deal with the nature of thought.

Place of Physiology.—Where does physiology appear in such a scheme? Physiology is not one subject but a synthesis of subjects which deals with the behavior of organisms, and as such it includes parts of biology, chemistry, physics, and geology. It is thus a *region* and has been indicated (Fig. 1) by the dotted line. Since physiology is so inclusive it requires a broader background of

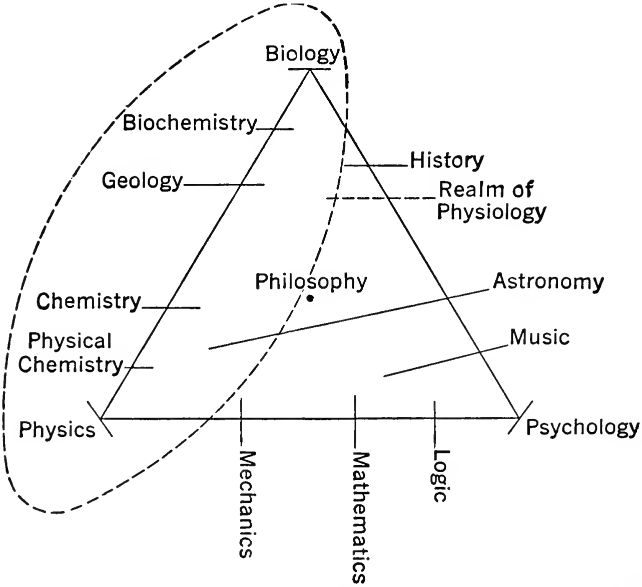

FIG. 1.—Illustrating the relation of the sciences to each other. (After Whetham.)

knowledge than many of the other sciences. The physiologist must be a chemist, physicist, mathematician, and biologist all in one.

A Liberal Education.— Let us suppose we are placed in a room which contains one window with variously colored panes of glass in the fashion described above. The colored sections affect both the color of the room and the landscape as seen through them. The room will be a mixture of different colors with no apparent pattern, and the world outside will likewise be of different colors as it takes on the color of the pane through which it is viewed.

But now, thanks to our university organization of studies, we are able to cover all the panes except the one we wish to use and to see the world for the time being through any one of the colors we choose. Obviously, both the method of selection and the use of all the panes have advantages and disadvantages. The world seen through the eye of a biologist seems all biology; through that of an historian, all history. The student must aim "to see life steadily and see it whole." This means that all lights must be used and as many sciences must be studied as possible. As Roger Bacon realized several centuries ago: "All the sciences are connected and foster one another with mutual aid. They are like parts of the same whole, every one of which accomplishes its own work, not for itself alone but for the others also."

Our allegory cannot be taken too seriously. Physicists do not see things red, biologists are not green, nor are the psychologists always blue. Neither is life a mere pane of glass to look through. There are, however, certain lessons which we may profitably learn from this before commencing the study of plant physiology.

1. We must remember the importance of philosophy as the mother of the sciences. All of them have come from her and then disowned her when they grew up; children often have this way of neglecting the old folks in our very modern period. As late as forty years ago physics was called "natural philosophy."

2. The necessity of a broad education can here be seen. We cannot know a thing well if we see it from only one point of view. Intense specialization should not commence until after the four years of general college training. Although it has been said that "the man of science must believe in a real world and to mix metaphysics and experimental science is fatal," it does not follow that the student of either science or metaphysics will be harmed by knowing a little of the other.

3. Each must learn to appreciate the labor of workers in other fields besides his own. Scientific men are often ignorant in general affairs, and likewise the humanist frequently knows nothing of the sciences. "Culture" at present seems to be based largely upon a knowledge of the humanities, which is unfortunate for the humanists because it makes their requirements so much easier to attain.

This lack of sympathy between the biological and physical sciences on the one hand and the psychological on the other is proverbial. When Faraday showed his invention of the dynamo to Gladstone, the latter was quite unable to appreciate it and asked like the good, practical man of affairs that he was: "But, what good is it?" "Why, sir, presently you will be able to tax it," replied the disgusted inventor. Taxation was something Gladstone understood. One only has to look at the present treatment of scientific matters in the general press to see how hopelessly ignorant of science are the average reporter and editor, and as a sample of literary "science" at its worst (or best) one might read Amy Lowell's "Sugar," in which she refers to sugar beets that "Red as the eye of cats in firelight, . . . fatten sugar in a crimson coat." Such "free verse" seems also to be free from any connection with the truth.

Why Study Science?—We study a science to accomplish the three following ends:

1. To learn a certain group of facts. The function of this book is to present the elements of plant physiology.

2. To learn something of the methods by which these facts have been obtained. This phase of the work is handled largely in the laboratory but is supplemented by reading the history of the development of science.

3. To stimulate the creative imagination. When new problems arise we should be able to plan experiments or tests which will help us solve these new difficulties and thus enable us to answer our own questions. The possession of a creative imagination is a prerequisite for doing original research in any field of knowledge.

THE BOTANICAL SCIENCES

The biological sciences are divided into two great divisions,—zoölogy and botany. Zoölogy deals with animals while botany is the science which treats of plants. Having thus defined the general scope of the subject, we can then divide our field very easily into the *pure* and the *applied* botanical sciences. Pure botany is the study of plants with no regard to their relations to man but from the point of view of the plant alone. That is, in pure botany all practical considerations, all benefits to man, are out of the question; it is botany for botany's sake. Applied or economic botany, on the other hand, is the study of plants with a view to applying the knowledge gained to human needs. No one would be so bold as to say that these lines are drawn sharply in practice or even that they should be, but in theory these are the limits. "Pure" botany is also an ill-chosen term because it seems to imply that there is something more ennobling, more scientific, and more honorable about the pure sciences than the applied branches. Fortunately the number of scientists who consider a thing bad because it is useful, is rapidly diminishing.

To the subject of pure botany there are two great divisions. First there are the subjects which deal *primarily* with structure or composition, and secondly, those which deal with function or operation,—the one group chiefly concerned with plant *statics*, the other with plant *dynamics*.

Morphology.—The structural subjects also fall into two categories. First one may consider the group of subjects which is concerned with structure *per se*, and then the group which is concerned with structures because of the light these studies throw upon the problem of evolution. The first group makes up what is known as *morphology;* while in the second category we have the branches of *systematic* botany, whose business it is to reconstruct the family tree of the plant kingdom.

Morphology also may be divided into two groups of studies,—those which consider structures primarily in their relation to *time* and those which consider them primarily in relation to *space*. *Embryology* is hence the study of the progressive changes in form and structure during the development of a particular organism. To be sure, space relations must also be considered, but the emphasis is placed upon the time element.

Morphology from the point of view of spatial relations has three main divisions: *cytology*, *histology* or minute anatomy, and *gross morphology*. Cytology is the study of the cellular units of the organism. Some cytologists may insist that their subject is as much concerned with function as with form, and it cannot be denied that modern cytology is interesting itself very much in function, but the *main* interest is in form, and the functional aspects of their subject have been taken over by the physiologists and the geneticists.

Histology or minute anatomy is the study of the tissues and their relations to the cells on the one hand and to the organs on the other. At present histology courses are largely courses in technique rather than in the actual tissues themselves. Gross morphology is that branch of morphology which treats of the relations of the plant organs to the plant organism as a whole. A large part of elementary botany is gross morphology.

Turning now to systematic botany, we see that it also has two main divisions,—*taxonomy* and *paleobotany*. The former concerns itself with the classification of *extant* plants and endeavors to show by a study of their structures their relationships to each other in the evolutionary scale. Paleobotany, on the other hand, tries to solve the problems of phylogeny and evolution by studying *extinct* forms, which exist to-day only as fossils.

This serves to delimit the provinces of morphology and systematic botany, but there exists a special field connecting them in which the emphasis is placed with equal stress upon form *per se* and form for the sake of determining relationships. This field known as *comparative morphology* is hence a connecting link between systematic botany and morphology. The workers in this field have divided up the territory in accordance with the great divisions of the plant kingdom and call themselves algologists, mycologists, bryologists, etc., depending upon their special interests.

Functional Studies.—Among the subjects which deal with the functions of plants, there are two main divisions,—*physiology* and *ecology*. The former considers the function, operation, and behavior of plants when grown under experimentally controlled conditions in the laboratory, as contrasted with ecology, which, in general, considers the behavior of plants out of doors in their natural environment, but here also the two fields overlap considerably.

This much is sufficient to define in general terms the great divisions of the so-called "pure" botanical sciences, but just as we have seen that morphology and systematic botany are connected by a series of intermediate subjects, in like manner there exist special studies connecting physiology with systematic botany and with morphology, where both phases of the work are equally emphasized.

Plant geography which considers the distribution of species as determined by heat, illumination, moisture, etc., is thus a connecting link between ecology and systematic botany. In a similar manner *experimental morphology*, which attempts to show the relations existing between form and function, is obviously a connecting link between the great divisions of physiology and morphology.

Applied Botany.—Turning now to the applied phases of botany, we see that plants are studied with a view to improving man's condition in six large fields,—agriculture, domestic science, forestry, medicine, brewing, and landscape architecture. *Agriculture* is largely concerned with the increasing of the world's supply of food and clothing. This requires a consideration of animals as well as plants but, since all food and most textiles come directly or indirectly from plants, as will be shown in Chapter III, agriculture is primarily a botanical science. To date there are five branches of agriculture which deal with plants, viz., agronomy, bacteriology, genetics, horticulture, and plant pathology.

Domestic science, which is concerned with the maintenance and care of the home, contains two divisions that are especially concerned with plant forms,—baking and food conservation. Since the yeast organism used in baking is a plant and since the causes of food decay are mostly micro-plants, it is easy to see that we are here concerned with botanical sciences.

Forestry is the science which deals with the production, conservation, and use of the world's supply of wood. Since wood is produced only by plants, forestry is a botanical science. Its divisions, silviculture and wood technology, are defined along with those of agriculture in the summarized outline at the end of this chapter.

Medicine is the science of keeping the human body in good health and in the best possible condition. Inasmuch as at the present time man's greatest enemies are the pathogenic bacteria, and since at least 75% of the curative medicines administered are derived from plants, it is easy to see that medicine is indirectly, if not directly, a botanical science. Two of its divisions,—bacteriology and pharmacognosy—are directly concerned with botany.

Brewing and the manufacture of fermented, alcoholic drinks of various kinds, of which wine and beer are the better known, are also important industries based upon the activities of microorganisms, chiefly yeasts of various types. Furthermore, the alcohol contained in the stronger liquors (gin, whiskey, brandy, etc.) is obtained by the like action of these same microörganisms. The entire industry of alcoholic beverages is, therefore, based upon the activities of plants, not only because yeasts are plants but also because plants furnish the raw materials upon which these organisms act and from which the alcohol is derived.

Landscape architecture is the science or art of improving the surface of the earth to better suit man's æsthetic nature or his love of the beautiful. It is hence a connecting link between the botanical sciences and the arts. Two of its branches, floriculture and landscape gardening, are directly concerned with plants. The landscape gardener must know something of soil requirements, season of blooming, rate of growth, etc., of the plants he uses if he is to obtain the most satisfactory results. In other words, he should be a botanist.

Since petroleum and coal come from plants, the huge industries based upon them, while not directly botanical, are *indirectly* dependent upon plant life in past geologic ages, and might also be included here. If not botanical industries, they are paleobotanical.

The following summarized key shows the relations of the botanical sciences to each other:

I. Pure botany—science for the sake of science.
  1. Structure dominant idea.
    A. Evolution not main interest—*Morphology.*
      a. Time relations dominant—*Embryology.*
      b. Space relations dominant.
Comparative   1. The cells—*Cytology.*
Morphology.   2. The tissues—*Histology* or *Minute Anatomy.*
        3. The organs—*Gross Morphology.*
Experimental   B. Evolution main interest—*Systematic Botany.*
Morphology   a. Extant plants—*Taxonomy.*
Pl. Geography.   b. Extinct plants—*Paleobotany.*
  2. Function dominant idea.
    A. Plants under controlled conditions—*Physiology.*
    B. Plants under field conditions—*Ecology.*

II. Applied or economic botany—science for the sake of man.
  1. Production of food, etc.—*Agriculture.*
    A. Field crops and relation of plant to soil—*Agronomy.*
    B. Soil bacteria—*Agricultural Bacteriology.*
    C. Plant breeding—*Genetics.*
    D. Garden and orchard crops—*Horticulture.*
    E. Plant diseases—*Plant Pathology.*

2. Management and care of the home—*Domestic Science.*
    - A. Baking.
    - B. Food preservation.
3. Production and conservation of wood—*Forestry.*
    - A. Forest management—*Silviculture.*
    - B. Use of wood—*Wood Technology.*
4. Maintenance of health—*Medicine.*
    - A. Pathogenic bacteria—*Bacteriology.*
    - B. Medicinal plants and drugs—*Pharmacognosy.*
5. Production of alcohol and alcoholic beverages.
    - A. Production of beer—*Brewing.*
    - B. Production of wine—*Vinification.*
6. Beautifying the surface of the earth—*Landscape Architecture.*
    - A. Cultivation of ornamental plants—*Floriculture.*
    - B. Harmonious arrangement of plants, etc.—*Landscape Gardening.*

It has been sufficiently emphasized that all phases of knowledge are related and that it is impossible to study one without touching upon the others. Such a summary may serve both to acquaint the student with the wide ramifications of the subject of botany and to show the position of plant physiology in reference not only to the other botanical sciences but to the general world of thought as well.

QUESTIONS

1. Why does a physiologist need to know chemistry and physics?

2. Were physicians or farmers first interested in the scientific aspects of botany? Why?

3. What is the connection if any between botany and (a) geology, (b) meteorology, (c) music?

4. Name five great industries based primarily upon plants.

5. To what extent are the following groups of men botanists: (a) farmers, (b) cabinetmakers, (c) physicians, (d) druggists?

6. In what field of botany is the man working who classifies and names the species and varieties of cotton?

7. If he studies the origin of the cellulose of the cotton fiber, in what branch of botany is he working?

8. If he studies the diseases of cotton, what kind of a botanist is he?

9. On what problems might a geneticist work in connection with cotton?

10. What kinds of botanists are employed by the United States Government? What are some of their problems?

11. Give the derivation of the names of the botanical sciences and discuss their fitness.

12. Is the study of the ascent of sap in trees a problem in physiology or ecology?

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CHAPTER II

THE CELL; THE COLLOIDAL CONDITION

Who hath despised the day of little things?
—ZECHARIAH 4:10.

Robert Hooke.—As a house is made up of units of concrete blocks, stones, or bricks and the various parts are connected by electric wires, plumbing conduits, gas pipes, water pipes, and other fixtures, so in a similar manner are plants composed of units differing in structure and function. These units are called *cells.* The discovery of the cell is generally attributed to Robert Hooke (1636–1703), an Englishman, who was at the same time a professor of geometry, an architect, an experimenter with flying machines, and a worker in optics. He improved the compound microscope which had been invented about 1590 by Zacharias Jansen of Middleburg, Holland, and looked at many objects with this new improved instrument. Among other things, he examined a piece of cork from the outer bark of a tree, and was much impressed by the fact that it was composed of many cavities separated by walls, giving the appearance of a honeycomb. He says, in describing his observations (1660–1665):

> I took a good clear piece of Cork, and with a Pen-knife sharpen'd as keen as a Razor, I cut a piece of it off, and thereby left the surface of it exceeding smooth, then examining it very diligently with a *Microscope,* me thought I could perceive it to appear a little porous; but I could not so plainly distinguish them, as to be sure that they were pores, much less what Figure they were of; But judging from the lightness and yielding quality of the Cork, that certainly the texture could not be so curious, but that possibly, if I could use some further diligence, I might find it to be discernible with a *Microscope,* I with the same sharp Pen-knife, cut off from the former smooth surface an exceeding thin piece of it, and placing it on a black object Plate, because it was it self a white body, and casting the light on it with a deep *plano-convex Glass,* I could exceeding plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular; yet it was not unlike a Honey-comb in these particulars.
>
> First, in that it had very little solid substance, in comparison of the empty cavity that was contain'd between as does more manifestly appear by the Figure. . . .

> Next, in that these pores, or cells, were not very deep, but consisted of a great many little Boxes, separated out of one continued long pore, by certain *Diaphragms*, as is visible by the Figure B, which represents a sight of those pores split the long-ways.

Unfortunately for the name which Hooke gave to the cavities he saw, cork is *dead*. The living substance known as *protoplasm* is no longer found in cork, and this living material is by far the most important part. Even when Hooke examined the thin slices of living plant tissue he failed to see the cell contents because of their transparency and the imperfections of his microscope. The name cell, however, stuck, and even when it was learned that Hooke had never seen the contents of his "cells," the name he gave was extended to include both the chamber and its contents. In fact, according to modern usage, there are many cells without any walls whatever.

While Hooke is generally given credit for discovering cells, it must be admitted from the drawings seen in the Roger Bacon manuscripts that this thirteenth century Oxford savant by the aid of some microscope of his own invention had seen cells four hundred years before Hooke.

Malpighi and Grew.—Hooke made no systematic study of plant structures. He was as a child in a strange room who runs from object to object as fancy dictates. In fact Hooke was much more interested in his microscope than in the structures he studied with it. The first systematic study of plant parts was made by Marcello Malpighi (1628–1694), an Italian professor of medicine, who ten years after Hooke's discovery published an *Anatomy of Plants*, and by Nehemiah Grew (1641–1712), an English physician, who, after working on the form and arrangement of the cells in various plant tissues, also published an *Anatomy of Plants.* But neither of these men, who are called the "fathers of plant anatomy," ever saw the protoplast with its living substance in the cells which they studied.

Protoplasm.—During the eighteenth century interest centered more in plant naming and classification than in plant structures, and it was not until 1772 that protoplasm was seen by Corti. Various observers had noticed this colorless slimy substance in the cell cavities, but it was not considered important until 1835 when Dujardin noted its constant occurrence in certain animals and recognized it as the living material. Purkinje (1839) called it protoplasm, and in 1846 Von Mohl applied the same name to the material in plant cells.

The Cell Theory.—Mirabel in 1808 showed that all plants are made up of cells, and Lamarck in 1809 extended this idea to animals, but the credit for the idea that every living plant and animal, however different in appearance, is composed of cell units is generally given to the botanist, Schleiden, and the zoölogist, Schwann (1839), because Mirabel and Lamarck both emphasized the cell wall rather than the cell contents. As Schwann stated it: "The elementary parts of all tissues are formed of cells in an analogous, though very diversified manner, so that it may be stated that there is one universal principle of development for the elementary parts of organisms, however different they may be, and that this principle is the formation of cells."

Recent research, however, has shown that before these two Germans published on the "cell theory," the French physician and botanist, Dutrochet (1776–1847), had (1) established the anatomical individuality of cells, (2) described the universal cellular structure of living plant and animal tissues, and (3) stressed the importance of the cell as the physiological unit of the organism. To him, therefore, goes the delayed gratitude of biologists for the establishment of the cellular nature of organic structures.

The Cell.—The plant cell is generally limited or bounded on the outside by a cellulose wall which is formed by the activity of the protoplasm within. On the inside of the wall, lining it and filling more or less of the entire space, depending upon the age and nature of the cell, is the viscid, nearly transparent, *living* protoplasm. In most cells is at least one nucleus, a round, more refractive body, which was first recognized by Robert Brown in 1831 to be of paramount importance as the center of cellular activities.

In 1861 Max Schultze showed that the substance which Dujardin had described and others had named was the same in plants and animals. He then formulated what has been called the "protoplasm doctrine," which states that the important physiological and morphological unit of all organized life is the nucleated mass of protoplasm.

Cell Parts and Inclusions.—The protoplasm surrounding the nucleus is now called *cytoplasm*. It may contain various organized bodies, among which are the *plastids*, the most important of which are the green chloroplasts. Within the cytoplasm may also be seen, with properly stained material, other bodies called mitochondria or chondriosomes. It seems probable that some of these chondriosomes may be the originators of the plastids and develop into them.

Within the cell, apart from the protoplasm itself, may be various amounts of water with salts, sugars, and other substances in solution. These may be materials which are to be used up in later constructive processes or waste products which have resulted from previous operations. This solution is called *cell sap*, and the regions containing it are called *vacuoles.* The cell sap in addition to matter in solution may also contain crystals of various kinds such as calcium oxalate as well as some starch grains and other insoluble materials.

Although Lepeschkin (1930) doubts their importance (and even their existence in many cases), most observers are agreed that the nucleus, plastids, etc., as well as the cytoplasm, have membranes on their inner and outer surfaces, which permit certain substances to pass through while keeping others from doing so. Such membranes are said to be *differentially permeable*, and the reactions which take place at such surfaces are extremely important in regulating the activity of the cell. The cell is able to carry on many different processes at the same time owing to these various membranes which surround the different structures in it, but the cell is nevertheless the physiological unit of the organism just as it is the morphological one. *The sum total of the activities of the organism is the sum total of the work of the cells.*

THE COLLOIDAL CONDITION

Graham.—Thomas Graham, an English physicist, who was working on the diffusion of substances through parchment paper (1861–1864) noticed that certain materials such as salts, acids, sugar, and the like easily diffused through the parchment membrane; while starch, albumin, gelatin, etc., did not pass through. He says: "As gelatine appears to be its type, it is proposed to designate substances of the class as *colloids* (Gr. *kolla* = glue) and to speak of their peculiar form of aggregation as the *colloidal condition of matter.* Opposed to the colloidal is the crystalline condition."

Although Graham speaks of the "colloidal condition" he seems to have regarded colloids as quite distinct from crystalloids and to have thought that the distinction was one of *nature* rather than of *condition.* He also appears to have believed that colloids were always organic.

The Colloidal State.—Since Graham's day much work has been done on colloids and it is now clearly established that "colloid" means a *state of matter* and not a peculiar *kind of substance.* Egg albumin and hemoglobin may be obtained in both the colloid and the crystalloid state. Gallic acid gives a colloidal solution in water and a crystalloidal solution in glacial acetic acid. Colloids are substances in which the particles of the material are in a finely subdivided state varying in diameter from about 1 μ to 1 mμ. (A micron or μ is one-thousandth of a millimeter; a mμ, or millimicron, is one-thousandth of a micron or a millionth of a millimeter.) Hence a colloid is matter in a particular *state* of subdivision and is not the name for an especial kind of matter.

If one started with a piece of silver and divided it up into smaller and smaller pieces, he would finally get pieces so small that, when stirred up in water, some time would be required before they settled out. But such pieces are not colloidal; they are merely forming mechanical suspensions. If these pieces are divided still finer there will come a time when they are too small to settle out when stirred up and yet they are much larger than molecules of silver. Such a mixture of silver and water is a colloidal solution.

Phases and Colloidal Mixtures.—In such a colloidal mixture a solid is suspended in a liquid, and there is said to be present a solid and a liquid *phase.* The liquid, in this case, is continuous and the solid is dispersed in the liquid. The liquid is then spoken of as the *continuous* phase and the solid as the *dispersed* phase. It is thus obvious that with the three states of matter—solid, liquid, and gas—there are the following eight possibilities for colloidal mixtures:

1. Continuous phase a gas.
    a. dispersed phase a solid,—smoke or very fine dust.
    b. " " liquid,—fog or mist.
2. Continuous phase a liquid.
    a. dispersed phase a solid,—*suspensoids.*
    b. " " liquid,—*emulsoids,* milk.
    c. " " gas,—foam.
3. Continuous phase a solid.
    a. dispersed phase a solid,—certain alloys, ruby glass.
    b. " " liquid,—opals and pearls.
    c. " " gas,—pumice stone.

True solutions on the one hand and mechanical suspensions on the other differ from colloidal solutions in the following particulars:

True solutions	*Colloidal solutions*	*Mechanical suspensions*
Particles are:		
1. of molecular size less than 1 mμ in diam.	1 μ–1 mμ in diam.	larger than 1 μ in diam.
2. invisible	visible under the ultramicroscope.	visible with the microscope or unaided eye.
3. able to pass through filters and parchment membranes.	able to pass through filters but not through parchment.	able to pass through neither filters nor parchment.
4. in molecular motion.	in Brownian movement.	in gravitational motion.
The system:		
5. shows high osmotic pressure.	shows low osmotic pressure.	shows no osmotic pressure.
6. is transparent.	shows the "Tyndall effect."	is generally opaque.

Of the various combinations mentioned above, the most important from a physiological point of view are the emulsoids and suspensoids. Protoplasm under normal conditions seems to be a watery solution with liquids and solid particles contained within it. It is hence a colloidal solution of suspensoids and emulsoids.

Emulsions.—In addition to these various types of colloidal suspensions, mention should be made of emulsions, which consist of drops of oily material dispersed in water or of drops of water dispersed in oily material. Mayonnaise, milk, and egg yolk are examples of such mixtures. If the particles are of colloidal size, the system then becomes a colloidal one, although this is seldom the case. Protoplasm contains droplets of oily material; it hence has some of the properties of an emulsion and is commonly spoken of as such. Under certain circumstances the inner phase of an emulsion may become the outer one, in which case the phases are said to be reversed. In cream, fat particles are dispersed in a watery liquid, but in butter the fat is on the outside and the water has become the dispersed phase.

Lyophobe and Lyophile Colloids.—If the colloidal, dispersed phase has little affinity for the material of the continuous phase, the colloid is said to be *lyophobic;* if there is much attraction between the two, it is called *lyophilic*. Since in biological studies, the chief dispersing medium is water, we may use the terms *hydrophobic* and *hydrophilic*. Most of the colloids of interest to biologists, such as proteins, starches, etc., are hydrophilic.

Sols and Gels.—Liquid colloidal systems are commonly called *sols* and, therefore, include the suspensoids and emulsoids mentioned above, as well as any colloidal system which seems to have the qualities of a solution and can be poured from one container to another. Colloidal systems, on the other hand, which are more or less rigid and behave like solids are called *gels*. Because of its peculiar structure a gel may appear solid and yet contain only a small amount of solid matter. Thus 1 g. of agar-agar can cause 99 g. of water to set to a rigid formation. Although a plant may be mostly water, it may nevertheless be firm owing to this property of gels. True gels may be formed from lyophile colloids as seen in the case of custards, gelatin, fruit jellies, heated egg white, muscle tissue, etc. False gels or *coagula*, on the contrary, are precipitates of lyophobe colloids and are of less importance in biological phenomena.

Colloids in Physiology.—Colloids are of the highest degree of importance in physiology, and among their properties which make them so important are (1) the ability of many of them to take up large quantities of water as cited in the preceding paragraph, (2) the large surface involved, and (3) the possession of electrical charges.

If a cube 1 cm. on an edge is measured it will be seen to have a total surface of 6 sq. cm. If this cube is then divided into cubes of 0.1 cm. on an edge, there will be 1,000 such cubes and a total surface of 60 sq. cm. (6,000 faces × 0.01 sq. cm. which is the area of one face). The following table shows the increase in surface as the colloidal state is approached and the number of cubes increases:

Size of cubes (edge in cm.)	*Number*	*Total surface (sq. cm.)*
1	1	6
0.1 (1 mm.)	1,000	60
0.01	1,000,000 (10^6)	600
0.001	10^9	6000
0.0001 (1 μ)	10^{12}	60000
0.00001	10^{15}	600000

This means that a little cube of starch 1 cm. on an edge when broken down into particles of colloidal size as found in starch paste, would have as much surface exposed to the solution as is found on a table 10 meters long and 6 meters broad!

Willard Gibbs of Yale University showed that in any system, according to the second law of thermodynamics, the amount of free unbound energy which could be used for doing work is always getting less and less as it approaches a minimum, while the bound energy which can no longer be used (or entropy) is progressing towards a maximum. Surface tension is a form of energy and, in accordance with this principle, anything which will lower the surface tension of a solution will do so and will collect at the surface. The large surfaces present on colloids give enormous room for these changes in surface energy to occur, and it is probable that the peculiarities of the plasma membrane and of the surface layers of all the structures of the cell are due in part to the phenomena which take place at surfaces.

The Electrical Charge.—Colloids are generally charged positively or negatively, i. e., they carry either positive or negative electrical charges. Most colloids such as colloidal gold, platinum, caseinogen, charcoal, and clay are negatively charged when in water, but others like the hydroxides of aluminum and iron are positive. Particles of protoplasm seem to be negatively charged.

The charge which a colloid carries is directly related to the fact that it may be precipitated out of solution by salts or electrolytes. Solutions which contain charged ions will precipitate electrolytes of an opposite charge. In a similar way a colloid may be precipitated by a colloid of the opposite sign. If a solution contains colloids of the same kind of charge, the repelling effects of the similarly charged particles will help to keep the particles suspended in the solution; but if colloids of an opposite sign are put into the solution, the charges will be neutralized and neither one will continue in suspension. Suspensoids are more sensitive to electrolytes than emulsoids.

Summary.—An elementary text is not the place for a complete discussion of colloids, but enough has been said to show their importance in any study of physiological phenomena. Protoplasm is a colloidal sol mostly of liquids in liquids, but there are also fibrous and other solid particles present, giving it some of the properties of a suspensoid; and it may at certain times even be a gel. It certainly possesses some gel characteristics, among which are its elasticity and rigidity. In all of the problems to be discussed in the following pages, these properties must ever be borne in mind; otherwise an intelligent study of the cell and its behavior is impossible.

QUESTIONS

1. Why are plant cells with walls and animal cells generally without?

2 What are the advantages of a cellular structure?

3. Are any cells of a plant useful when dead?

4. Should Hooke really be given credit for discovering the cell? Why?

5. Why had cells not been discovered by the Greeks?

6. Why are emulsions, suspensions, and gels most important of all the colloidal mixtures for a physiologist?

7. Among these mixtures, why is a gas within a gas not mentioned?

8. A plant is 40–95% water. Why is it able to retain its shape and form?

9. An *Elodea* leaf is about 1 cm. long and 2 mm. wide. If the leaf is 100 cells long and 50 cells wide, and if each cell is square in cross section, what is the total cell surface exposed by one layer of cells?

10. What are the laws of thermodynamics?

11. Define electrolyte, caseinogen, and surface tension.

12. Discuss the construction and use of the ultramicroscope.

13. What is the "Tyndall effect"?

14. What is Brownian movement?

15. Who was Francesco Selmi and what was the relation of his work to that of Graham?

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CHAPTER III

PHOTOSYNTHESIS: GENERAL

All flesh is grass.
—ISAIAH 40:6.

Plant Analyses.—If an ordinary green plant is placed in an oven, it is found that a large percentage is water, which can thus be driven off. Of the *dry matter* which is left after the water has been expelled, a certain percentage can be burned. This combustible portion passes off in gases and smoke leaving a small incombustible residue known as the *ash*. The mineral matter or ash is seldom over 5% of the total plant, leaving 95% to be divided between the water and the combustible materials, the proportions of which vary in different types of plants as follows:

Plants	*Per cent water*	*Per cent combustible matter*
Woody plants	40–50	55–45
Herbs	65–75	30–20
Succulents	80–90	15– 5
Algæ and water plants	85–95	10– 1

Of the combustible dry matter, about 45% is carbon contained in various carbon compounds within the plant. Organic chemistry, which now treats of carbon compounds, was called *organic* chemistry because it was once thought that these carbon compounds were formed only in living organisms. Since the days of Wöhler (1828), however, organic compounds have been synthesized in the laboratory in an ever-increasing variety and number. Many are now synthesized and studied which have never been found in plants or animals, so that the field of organic chemistry is much larger than its name would indicate and the name has hence become a misnomer.

Source of Combustible Materials.—These organic compounds made in the plant are manufactured from simpler inorganic materials, which the plant takes from its environment. These organic materials are then used by the plant in its life processes and ultimately returned to the inorganic environment, either during the lifetime of the plant or after it is dead, as the result of

Dr. Rodney H. True, Professor of Botany, University of Pennsylvania, Philadelphia. *Mineral nutrition of plants; Toxic action of solutions; Turgor; Growth and temperature.*

Dr. F. E. Lloyd, Professor of Botany, McGill University, Montreal, Canada. *Transpiration; Physiology of stomata; Fluorescence.*

the processes of decay. This continuous transfer of materials from the organic to the inorganic realm and vice versa results in an organic cycle, which is represented diagrammatically in Fig. 2.

Foods.—A very large percentage of the organic materials are built up into what are called *foods*, which have two main purposes: (1) they supply energy when burned and (2) they supply material used in construction. There are three classes of foods which are structurally different and which fulfill the different needs of the plant,—the *carbohydrates*, *fats*, and *proteins*.

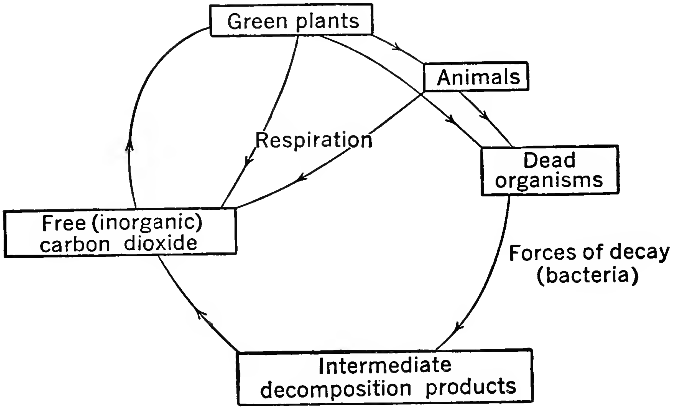

FIG. 2.—Illustrating the organic carbon cycle.

The simplest of these are the carbohydrates, of which the starches and sugars are the most important. They are composed of carbon, hydrogen, and oxygen and, when burned in the plant, liberate energy and heat the same as when burned in an animal or in a stove. In the plant, carbohydrates also play an important rôle in construction. The cellulose cell wall so characteristic of plants is a carbohydrate. Animals, on the contrary, use very little carbohydrate except for supplying energy.

Fats are used in the plant almost exclusively as sources of energy and very little for construction. Proteins are used largely in the manufacture of protoplasm and little, if at all, as sources of energy. Both the fats and proteins will be discussed more fully in a later chapter.

Source of All Food.—All of the foods for both plants and animals are built up by green plants. *The green plant is the source of all food*, and this truth is one of the most outstanding facts in all nature. Not only the food which plants themselves use, but the food of all animals as well, including man, comes from plants. Carnivorous and omnivorous animals get all their food directly or indirectly from plants.

This process of building food starts with the manufacture of the carbohydrates,—the sugars and starches; and this construction of simple carbohydrates from inorganic materials such as water and carbon dioxide is called *carbon assimilation* or *photosynthesis.* The term "photosyntax" was proposed by Barnes in 1893 and later changed to photosynthesis, which is the term now commonly used in America, although in England and other European countries, the phrase "carbon assimilation" is still in common use.

Photosynthesis.—Photosynthesis is the *manufacture of simple carbohydrates from carbon dioxide and water by the chlorophyll of the green plant in the presence of light.* Photosynthesis is hence the most important organic process in nature because upon it all the other life processes depend. For this reason it has been called "the most fundamental fact in science."

Historical.—Bonnet (1769) noticed bubbles coming from grape leaves immersed in water. From boiled water no bubbles were obtained, but the phenomenon remained without explanation.

Priestley (1771) noticed that mice living in a confined space gradually vitiated the air and made it unfit for continued life. He sought some method to restore the air to its original purity and placed some plants under a bell jar under which the mice had previously been. He found that after some time the air was improved and again capable of supporting animal life but, unfortunately, he was not always able to repeat the experiment, and he could not understand why sometimes the air was improved by the plants and sometimes not.

Ingen-Housz, a Dutch physician (1779), showed that this purifying process was effected only by the *green* parts of plants and only in the *light.* He did not know why plants should have this effect nor did he have any idea concerning the importance of the matter in the nutritional economy of the plant, but arrived at the conclusion that plants had two kinds of "respiration,"—nocturnal and diurnal. Influenced by the philosophical ideas of his time, he thought that this was a purposeful reaction for improving the air for the benefit of man Neither did Ingen-Housz know precisely what gas was removed from the air, and he even thought that air which had been made impure by too much hydrogen resulting from the contact of metals with acids could be improved by plants in the same manner as that vitiated by animals.

It remained for Senebier (1782) to show that carbon dioxide was the only gas absorbed and that this absorption was related to the nutrition of the plant. He thus explained the results of Bonnet and correlated the work of all the previous investigators.

De Saussure (1804) then showed that as the carbon dioxide was removed, the same amount of oxygen was returned to the air, or that the CO_2:O_2 ratio was approximately 1:1. He also showed that, as a result of this absorption and decomposition of carbon dioxide, the weight of the plant was increased; and he demonstrated that in an atmosphere deprived of carbon dioxide the green plants would not live. To quote his own words: "The observations of Priestley, Senebier, and Ingen-Housz opened the path which I have followed but they did not reach the goal which I have tried to reach. . . . My researches have enabled me to demonstrate how much greater is the contribution of water and air to the formation of the dry material of plants growing in fertile soil than the materials which they absorb in the water through their roots."

That De Saussure and his followers minimized the importance of the roots in the absorption of carbon has been shown recently (1927) by Stoklasa who finds that plants absorb comparatively large amounts of carbon in the form of carbonates. This carbon is largely a respiration product of bacteria in the soil. Stoklasa finds that in some cases there are 100 pounds of carbon absorbed by the roots for every 154 pounds of nitrogen and every 106 pounds of potash. This seems a considerable amount, but also Livingston and Beall have called attention to the large amount of carbon dioxide which may be taken in by the plant dissolved in water absorbed by the roots. They calculated that with a high rate of water absorption and a high concentration of carbon dioxide in the soil solution, as much as 6–8% of the carbon dioxide decomposed by the leaves of an alfalfa plant might enter through the roots. Confirming these researches is the work of Bergamaschi, who found that plants with their roots in a medium rich in carbon dioxide were much more active in photosynthesis than those in a normal medium. Whether the carbon is absorbed as dissolved carbon dioxide or as carbonates, these researches have shown that it is necessary to revise somewhat our ideas of the importance of carbon absorption by roots.

Boussingault (1860–1890) studied all these problems in detail quantitatively, and to him we owe most of our precise information on these points. He carefully measured the carbon dioxide taken in and the oxygen given off and established their equality in volume. One very ingenious and interesting experiment was devised to show that the decomposition of the carbon dioxide into carbon and oxygen began immediately after the plant was illuminated. Carbon dioxide was mixed with hydrogen and nitrogen and exposed with the plant and a piece of phosphorus in an experimental chamber. As soon as light was allowed to fall on the chamber, white fumes of phosphorus pentoxide (P_2O_5) would appear indicating the presence of oxygen. As soon as the apparatus was darkened, no more fumes appeared and those already present would dissolve and disappear in a vessel of water contained in the chamber.

In this experiment the amount of carbon dioxide was much higher than is normally present in the air and the question arose as to whether the plant could actually remove the small amount of carbon dioxide found in air under ordinary conditions (about 0.03%). To settle this point Boussingault placed a plant in a jar through which a current of air was passed, and the air was analyzed before and after entering the jar. He found that the plant *was* able to take out the small quantity of carbon dioxide present, much to the astonishment of the workers in the laboratory, as narrated by Timiriazef:

> To what extent the precision of this experiment aroused the admiration of his contemporaries (as did most of Boussingault's researches) can best be shown by a story which Boussingault himself told me. "The experiment was carried on with Dumas, each worker making his own weights and records independently in order to secure more reliable results. At first all went nicely and the plants decomposed the carbon dioxide as they were expected to do. Then matters suddenly changed. On a bright, sunny day the plants began to *make* carbon dioxide instead of decomposing it. That evening we examined the results with much surprise and stared at each other in blank amazement. We remembered the misfortune which had attended Priestley when he tried to repeat his famous experiment. Then one fine morning several days later, Regnault, the famous physician, who had been watching our experiment with much interest, began to laugh at our long faces and admitted that he had been the cause of our trouble. Every day while we were out at lunch he had sneaked into our

laboratory and breathed into the apparatus 'in order,' he explained, 'to be certain that you were not taking an x for a y and could really determine such small amounts of carbon dioxide.' "

Analysis of Photosynthesis.—To facilitate our understanding of this most important process, the subject of photosynthesis will be analyzed and discussed under the following heads:

a. Methods of demonstrating.
b. The gaseous exchange—taking in of carbon dioxide.
c. Absorption of energy as light.
d. The use of the energy to synthesize elementary organic compounds.
e. The condensation of the synthate into more complex substances.
f. The gaseous exchange—the elimination of oxygen.

Only a and b will be discussed in this chapter, the others being reserved for the following chapters.

Demonstrations.—To demonstrate photosynthesis it is a simple matter to compare the dry weight of a plant in the morning and the evening. In the morning cut a hundred disks from the leaves of a healthy plant. Let the plant remain in the sunlight all day, and in the evening remove a hundred more disks from the same leaves. Dry in the oven and compare the weights of the two sets of disks. It will be found that those cut in the evening are somewhat heavier than those removed in the morning due to the weight of the food manufactured during the day.

An experiment similar to Boussingault's can be performed. The plant may be placed in a closed chamber with removable glass tubes attached so that a sample of the air can be easily taken for analysis at convenient intervals. If analyses are made in the morning and evening it will be found that during the day the carbon dioxide has diminished in amount and the oxygen has increased.

To show the evolution of oxygen, *Elodea* or other water plants may be used. Invert a funnel over the mass of plants and cover the end with an inverted test tube filled with water. If placed in the sunlight, bubbles of gas will be seen escaping from the plants and rising into the test tube where they collect and displace the water. When full of the gas, the test tube may be removed and the gas tested by a glowing splinter or twine. It will be found to support combustion and give all the tests for oxygen.

That carbon dioxide is removed from water by hydrophytes may be shown very nicely by placing in the water with the plants a little indicator such as phenolphthalein. As the carbon dioxide is removed the water becomes more alkaline, and it will hence take on a decided pink tinge if placed in sunlight.

Lastly, among the easy demonstrations should be mentioned the direct test for starch with iodine. Test plants for starch which have been kept in the dark and in the light. Those in the dark show little or no starch depending upon the length of time they have been left there, while those in the light turn blue when iodine is applied showing the presence of starch. If geranium or other leaves are tested at the end of one, two, and three hours after being brought into the light, they will show a progressively increasing amount of starch present.

Amount of Carbon Dioxide Consumed.—In 10,000 liters of air there are only 3.3–3.5 liters of carbon dioxide. This weighs about seven grams but only three-elevenths is carbon. In 10,000 liters of air one finds therefore only two grams of carbon. An ordinary recitation room is about 9 × 11 × 4 meters, or it has a volume of about 400 cu. m. This means 400,000 liters, which would contain about 80 g. of carbon or enough for 175 g. of starch. An average sized oak tree would hence deprive 12,000,000 cu. m. of air of carbon dioxide to get the requisite amount of carbon stored up in the cellulose of its woody tissues. This means a volume 30,000 times the size of the above-mentioned recitation room.

It has been computed that clover assimilates 63 mg. of carbon dioxide per sq. m. per hour and that squash leaves assimilate 25 g. of sugar per sq. m. of leaf surface in 15 hours; while the catalpa has been found to manufacture 3 g. of starch per sq. m. per hour. At this rate it would take about 25 sq. m. of leaf surface to use up the carbon dioxide given off by the average man in breathing during 10 hours, or if computed on the basis of 24 hours, about 60 sq. m.

Three hundred bushels of potatoes on an acre means about 2,500 kg. of dry matter including tops and roots. In terms of starch, this would mean that all the carbon dioxide would be used over the acre to the height of a mile and a third, assuming an even distribution of the gas. It has been estimated that during the growing season the average leaf produces enough sugar to cover itself a millimeter thick, and that the plants of the United States manufacture a cubic mile of sugar each year.

Also it has been calculated that the wheat plant produces annually seventy million tons of carbohydrate material, requiring one hundred fourteen million tons of carbon dioxide. One ounce of starch yields 116 calories when burned. Therefore, if all this wheat were burned, the heat produced would be sufficient to raise to the boiling point the water in an ice cold lake 4 miles long, 4 miles wide, and 40 fathoms deep!

Source of the Carbon Dioxide.—Such figures give one some conception of the enormous activity associated with photosynthesis, and when it is recalled how much carbon dioxide is taken from the air annually by all the plants in the world, it is little wonder that people refused to accept for a long time the conclusions of the early investigators. Where does all this carbon dioxide come from? What are the sources which keep this sea of carbon dioxide from being pumped dry by the photosynthetic action of the green vegetation?

First in quantity, but much more recent historically, is the carbon dioxide resulting from the combustion of wood, coal, gasoline, etc. When these products are burned, the carbon again combines with oxygen and returns to the atmosphere as carbon dioxide. Over 500,000,000 tons of coal are burned annually, which makes more than twice that much carbon dioxide. Secondly in importance is the carbon dioxide resulting from the respiration of plants and animals. In these oxidation processes within the body, the carbon and oxygen are united and given back to the air. An adult man gives off about 900 grams of carbon dioxide daily. A third important source is the decay of organisms, during which process carbon dioxide is given off to the air. Rocks, particularly limestones, contain large amounts of carbonates, which give off carbon dioxide to the air during the processes of weathering and disintegration. Finally volcanic action should be mentioned, since the gases given off during eruptions are rich in carbon dioxide.

Distribution of Carbon Dioxide.—All these various sources tend to restore the carbon dioxide taken from the air and to keep the amount present about the same. The distribution over the surface of the earth, owing to diffusion and the action of wind currents, is everywhere nearly equal, although, because of the fact that carbon dioxide is heavier than the nitrogen and oxygen which make up the bulk of the atmosphere, the air near the earth tends to be slightly richer in carbon dioxide than that at high altitudes. This, coupled with the fact that soil organisms give off large quantities of carbon dioxide, may produce a layer next the soil especially rich in carbon dioxide, which may be exceptionally favorable for plants near the ground.

The Regulatory Action of Water.—Since carbon dioxide is soluble in water, there exists an equilibrium between the carbon dioxide dissolved in the oceans, streams, and lakes and that present in the air above these bodies of water. Water generally contains a bit more carbon dioxide than the air above it, and, as the amount above increases, still more dissolves in the water and becomes combined in the form of carbonates with the ocean salts. If the air above diminishes below normal in the amount of carbon dioxide contained, the ocean gives up some of that dissolved and combined in it, thus serving as an autoregulator of the amount present in the air.

The Carbon Dioxide in the Coal Age.—In Carboniferous times (250 to 500 million years ago) when the coal was formed, there was evidently more carbon dioxide present in the air than now. The warmer temperature of the earth and seas would not permit so much to be dissolved in the oceans as at present, and there seems to have been a great excess in the air. This permitted the luxuriant growth of vegetation which covered the now temperate regions of the earth at that distant period. The excess of production over consumption and decay of that age, we are burning at the present time. The carbon molecules which were removed from the Carboniferous carbon dioxide by the Paleozoic chlorophyll are once more permitted to combine with oxygen and go back to the inorganic state from which they came so long ago.

QUESTIONS AND PROBLEMS

1. What was Wöhler's contribution to chemistry?
2. If a bushel of potatoes contains 35 lbs. of starch, how much of this is carbon?
3. How do plants and animals differ in their use of carbohydrates? Of what advantage is this to animals?
4. Many elementary texts state that plants differ from animals in that they (plants) use *inorganic* foods. Criticize this statement.
5. Explain the results of the demonstration experiments described on page 25.
6. Why is photosynthesis "the most fundamental fact in science"?
7. A good crop of corn is 75 bushels to the acre. If one-third of this is

carbon, how many tons of hard coal must be burned to replace the carbon dioxide removed from the air?

8. If a growing corn season is 100 days, a village of how many inhabitants will replace by their respiration the carbon dioxide removed from the air by a field of ten acres? Use the respiration data in the text.

9. How many tons of wood are in the oak tree mentioned on page 26?

10. How many liters of air are required to provide the carbon dioxide for a gram of sugar?

11. Explain how the equilibrium is maintained between the CO_2 of the air and the carbonates of the ocean.

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CHAPTER IV

THE DETERMINING FACTORS IN PHOTOSYNTHESIS

> Forever . . . shines that high light whereby the world is saved.
>
> —John Hay.

Light.—From what has been said in the previous chapter, it is not surprising to learn that light plays a highly important part in photosynthesis. The energy stored up in foods comes from the energy of light. As a dynamo is the machine through which the energy of falling water is transformed into that of electricity, so the green plant is a machine which transforms the energy of light into the energy of carbon compounds.

Light Quality.—In any discussion of light, both the *intensity* (brightness) and *quality* or wave length (color) must be considered. If pure white light is passed through a prism, it is split up into the seven colors of the visible spectrum with violet at one end and red at the other. If this same sunlight is passed through the prism of a spectroscope, about 750 dark bands or lines are found in the spectrum obtained. The most conspicuous of these were designated by Frauenhofer (after whom they were named) with the letters A–H and are used as convenient reference points in the spectrum. The wave lengths of the visible spectrum rays vary from 0.00076 mm. in the red end to 0.00039 mm. in the violet. Rays of corresponding greater and shorter wave lengths have been found in the invisible infra-red and ultra-violet portions of the spectrum. By *quality* is hence meant the wave length, of which the color is a function.

Now if a thermometer is placed in the various regions of the visible spectrum, the highest temperature is obtained at the red end between lines A and B. From here the temperature gradually diminishes towards the blue end, whereas it increases to a maximum in the invisible infra-red portion beyond line A. Chlorophyll is most rapidly decomposed in the orange-red region between lines B and C, and more light is also absorbed by chlorophyll in this region than in any other. Although this part of the spectrum is most important as far as chlorophyll is concerned, not all reactions are most intense here. The human eye is most sensitive to light in the region of line D; while photographic plates, because of the silver salts they hold, are most sensitive to the rays between G and H.

In 1864, Sachs found that plants placed under double-walled glass jars with a colored liquid between the two walls, made more starch under a red solution than under a blue one, which shows that the red rays are more important in photosynthesis than the so-called "chemical" rays of the blue end.

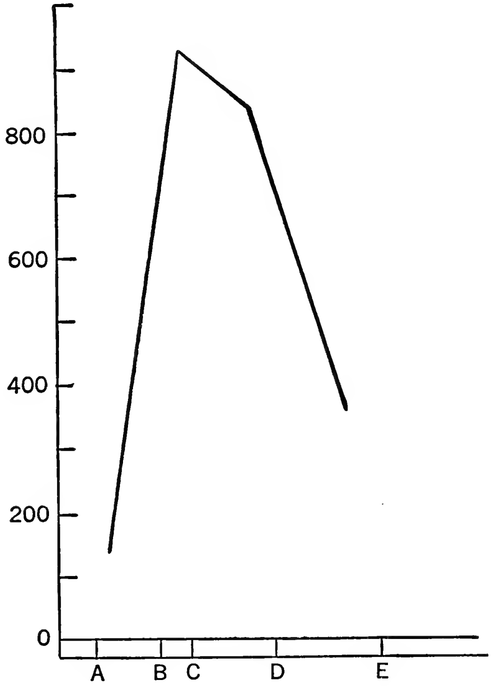

Fig. 3.—Graph showing relative rates of carbon dioxide decomposition in various parts of the spectrum. (After Timiriazef.)

Sachs, however, had not localized the action more closely than the red end of the spectrum. More exact experiments were carried out by Timiriazef (1875) who placed bamboo leaves in closed glass tubes with air containing 5% of carbon dioxide. These tubes were then placed in various parts of the spectrum, and at the conclusion of the experiment the gas was carefully analyzed. Fig. 3 shows his results. The greatest decomposition occurs between B and C, in the region where the most light is absorbed by the chlorophyll.

Engelmann (1882) also devised an ingenious experiment to show the region of greatest carbon dioxide decomposition. A filament of a green alga was placed in a culture of bacteria which are active only in the presence of oxygen and which do not move in its absence. After the bacteria had exhausted the oxygen present, the filament was placed in a solar spectrum and observed under the microscope. The bacteria were seen to move from the regions between D and F and to collect especially between B and D, with a smaller but noticeable movement towards the blue end in the region between F and G. It is chiefly in these regions then that oxygen is liberated or that carbon dioxide is decomposed. More recently Dangeard has used a modification of this method with *Chlorella* and *Scenedesmus.* Instead of using bacteria as indicators of the oxygen release, he counted the bubbles of oxygen directly as they were given off. These algæ respond more quickly than *Elodea* (Chap. III) and there is no inactive tissue to absorb and hold gases (thus resulting in errors) as in *Elodea.* In all such experiments, however, where a diffraction spectrum is employed, it must not be forgotten that the results obtained in the spectrum are not exactly the same as would occur in incident light, since the intensity per unit area in the different regions is a function of the index of refraction. That is, when different *qualities* are being compared, one must be sure that the *intensities* remain the same.

Timiriazef also determined the relative efficiency of the blue and red ends in decomposing carbon dioxide. In the blue end only 54% as much carbon dioxide is decomposed as in the red, but since the region of absorption in the blue end is nearly four times as broad, the relative efficiency per given area of the blue is only 14%. This is due to the difference in energy absorbed, as shown by the heat effect in the two ends of the spectrum. The blue light of the spectrum is absorbed by chlorophyll but is less efficient because it represents a smaller amount of energy. The relation between the photosynthetic efficiency and the energy absorbed is so close as to warrant the conclusion that *the photosynthetic work accomplished varies directly with the energy absorbed from the light regardless of the wave length.*

In ordinary green plants then the rays between B and C (the orange-red rays) are the most efficient in photosynthesis. But in other plants such as the algæ where other prominent pigments are present besides chlorophyll, the greatest activity may lie in a different region of the spectrum. In the blue-green algæ, the maximum activity is near D in the orange-yellow; in the brown algæ between D and E in the yellow-green and also between B and C in the red; and in the red algæ between D and E. Engelmann believed that the color of light most absorbed in photosynthesis was complementary to the color of the plant as shown in the following table:

Color of alga	*Light absorbed*
blue-green	orange-yellow
green	reddish-orange
brown (yellow)	red and yellow-green
red	yellow-green.

This is supported by the fact that the blue-green alga, *Oscillatoria,* undergoes chameleon-like changes of color (this, however, is not the origin of the name!) depending upon the light in which it is placed. Unlike the chameleon, however, it does not take on the color of the light falling upon it but rather that of the complementary color. Thus in blue light it is brownish-yellow, in green light it becomes red, in yellow light it turns to bluish-green, and in red light it assumes a green color. This alga also contains a reddish and a bluish-green pigment, and the complementary adaptation seems to depend upon the arrangement and the amounts of the various pigments in the cells.

At any rate this ability to absorb other rays besides red is extremely important for algæ growing in the ocean depths and is directly connected with their distribution. Blue-green and green algæ are found nearest the surface in comparatively shallow water. The browns are found deeper; while the reds occur at the greatest depth of all. The red light so important to green algæ penetrates the water only a short distance, while the yellow and green light so necessary for the brown and red algæ may penetrate to a much greater distance. It is interesting to note in this connection that red algæ when grown near the surface of the ocean are greener than usual, not because they contain more chlorophyll (the reverse is actually the case), but because they contain less of the red pigment.

Intensity.—In all such matters where quantity enters into consideration it is necessary to speak of the "cardinal points,"—

the minimum, the maximum, and the optimum. These terms are self-explanatory. The cardinal points differ with the species, some plants needing a great deal more light than others. Ferns, firs, beeches, and many other plants of the deep woods thrive best in not too bright sunlight and are called *heliophobous* (sun-fearing) plants while others such as the pine, birch, and poplar thrive best in bright sunlight and are called *heliophilous* (sun-loving).

Not only is there a great variation in the intensity of light in which plants thrive best, but an equally great variation exists in the *minimum* intensity of light required. Thus *Oxalis* can grow well in bright light, but it can also thrive in very weak light. *Sequoia sempervirens* can use light as weak as 0.75% of sunlight, but the Engelmann spruce and Douglas fir require a minimum intensity of 1.2–1.5% and the pines of 2–6% full sunlight. The fern, *Adiantum capillus*, was found in light 1/1700 as bright as sunlight; and the algæ of caverns can use light reduced to 1/2500 that of sunlight (Morton and Hofman, 1927).

Schistostega osmundacea, an extreme heliophobous type, is a moss which inhabits caves where it can grow in the very weak light which penetrates to it. Like most shade plants, it is very rich in chlorophyll, which may partly explain why it can thrive so well in dim light. But *Schistostega* possesses other advantages: the protonemal cells form a thin plate at right angles to the impinging light and each cell is lens-shaped so that the light is concentrated on the chloroplasts, which lie in the bottom of the cell. Thus what little light reaches the cells is well used.

It has been shown experimentally that the algæ of the ocean can utilize the light of moonlight, and the fact that during periods of full moon the ocean contains less carbon dioxide than during the dark of the moon substantiates these results. In fact, at equal intensities moonlight seems to be many times (100,000 times, according to some!) as efficient as sunlight. This has been explained as due to the fact that about 10% of the moonlight is polarized.

Light as a Limiting Factor.—According to Brown and Escombe (1905) light is seldom a limiting factor in photosynthesis except, of course, at night. For the average plant the light could be reduced to one-half of the intensity of bright sunlight without slowing up the rate of photosynthesis, and many plants thrive well which get only 2% of the available light. It seems probable, however, that heliophilous plants have an optimum light intensity which is somewhat higher than that for shade plants, even though Johansson has reported that the fern, *Dryopteris austriaca*, gave maximum photosynthesis in 30% full sunlight and *D. spinulosa* increased its photosynthetic activity up to 60% full sunlight,—amounts rather high for heliophobes.

Reinke (1884) provided us with a good illustration of the variation of growth with sunlight. He measured the production of young plants from an older stem of *Elodea canadensis* and found that the number of plants produced varied with the intensity of the sunlight as follows:[1]

Intensity of light:	1/16	1/8	1/4	1	2	4	8
Number of detached plantlets:	4	10	21	39	40	30	30

Here we see that diminishing the light to one-fourth normal reduces the growth only one-half, while doubling the light has practically no effect on the number of plants produced. High intensities of light diminish production through their destruction of the chlorophyll (see below).

In agreement with these general results, Boysen-Jensen found that in forest trees photosynthesis increased with increasing light until 1/20 full daylight was reached; increasing the light beyond this had no further effect. Lundegardh found that an increase beyond 1/10 full sunlight was without effect; but Stålfelt has insisted that light is frequently a limiting factor in the growth of trees. He found that pine and spruce absorbed more carbon dioxide as the light was increased up until full daylight. Similarly in fruit trees, he reports that in the sunlight they can use about 50% more carbon dioxide than when in the shade. It is possible that the reason for these differences lies in the fact that in Sweden "full daylight" is not as intense as in regions farther south.

Plants need for growth and maturation a definite number of light energy units, the unit being a product of the time and the intensity. Within certain limits, a bright light acting for a short length of time may have the same effect as a weak light acting for a longer time, if there is abundant carbon dioxide present; but as Davis and Hoagland (1929) have pointed out, as a result of their experiments with wheat, the plant probably functions more

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[1] Fraction of normal sunlight.

efficiently when the radiant energy is distributed over a longer period of time at a lower intensity than when the opposite condition holds. The ripening and growth of plants can hence be speeded up enormously by using artificial light and increasing the working day of the plant. For this reason orange growers of central California where the summer days are very long can market their fruit a month earlier than their competitors 400 miles farther south where the days are shorter.

Efficiency of the Leaf.—Brown found that a sunflower leaf received on a sunny day 600,000 gram-calories of heat energy per sq. meter per hour. During the same time the same area produced 0.8 g. of carbohydrates which requires 3,200 gram-calories for its manufacture. Thus the leaf was able to store up in the manufactured food only 0.5% of the solar energy which fell upon it. Considered as a mechanism for transforming solar energy into the chemical energy of organic compounds, it is a machine of very low efficiency. However, it is not so bad as it sounds. Of the light which falls upon the leaf 30–60% is reflected and transmitted and only 40–70% is absorbed by the leaf. If we use the higher figure of 70% actually absorbed by the leaf, of this a large fraction, or 60–65% of the total light falling on the leaf, is absorbed by the chlorophyll-free part of the leaf, leaving only about 5–10% of the total light falling upon the leaf to be absorbed by the chlorophyll-containing cells, while the *chlorophyll* itself probably gets no more than 3–4% of the total light. This may be summarized as follows: Of the total light falling on the leaf,

30% is reflected and transmitted.

70% is absorbed by the leaf.

60% is absorbed by the chlorophyll-free part of the leaf,—epidermis, cell walls, water, etc.

10% is absorbed by the chlorophyll-containing cells.

3.5% is absorbed by the chlorophyll apparatus and used in photosynthesis.

A green leaf compared with a white leaf of the same plant absorbs about 4% more light which agrees well with the above figure of 3.5%. Inasmuch as the chlorophyll actually absorbs only 3.5% we must now multiply our efficiency figure by 100/3.5 which shows the chlorophyll apparatus to be 15% efficient rather than 0.5%.

The remainder of the 70% absorbed by the leaf and not used in photosynthesis, i. e., about 66% of the total solar energy received, is spent in heating the leaf as well as the surrounding air. This might cause an increase in temperature of 10–15° C. if it were not for the fact that most of this heat is used in the conversion of water to water vapor within the leaf. For every gram of water vaporized, 536 calories of heat are employed, and the result is that with an abundant water supply, the leaf may be actually cooler than its surroundings. Green tomato fruits, on the other hand, not having the cooling facilities of leaves, may be nearly 10° C. warmer than red ones (Harvey, 1924).

Temperature.—According to the researches of Matthaei (1905), temperature is one of the most important of the external conditions which influence photosynthesis. While some conifers accustomed to very cold climates may make some food at −25° C. and even lower, the minimum for most plants is about −6° C., the optimum near 37° C., and the maximum about 45° C. Kreusler (1887) had previously reported the optimum at 25° C. but this difference may be explained by the difference in the materials used. For crop plants in the temperate zone it is generally safe to *assume* an optimum of about 30°, although it is better to *know* precisely for the individual species. Although Walther (1927) reported that in *Vicia* photosynthesis did not increase directly with the temperature but that there were maxima at about 10, 20, and 30° C. (with drops in between), Honert (1930) found that photosynthesis increased continuously with the temperature from 12 to 24° C. in good light. This is more in agreement with the general conception in regard to this matter.

In studying the effect of temperature upon a reaction it is often useful to know the *temperature coefficient.* If a reaction goes on twice as fast at 20° C. as at 10° it has a temperature coefficient of 2. According to Matthaei and others, the temperature coefficient of photosynthesis between 10° and 30° is 2, i. e., at 20° the process is twice as rapid as at 10° and at 30° twice as rapid as at 20°. Physical reactions generally have a temperature coefficient of less than 2 while chemical reactions commonly have a coefficient of 2 or more. The above results, therefore, indicate a chemical reaction rather than a physical one. Brown and Heise (1917), however, found the temperature coefficient to be 1 instead of 2 and hence concluded that they were dealing with a physical (photochemical) reaction. As will be seen in Chapter VI, these two points of view may be reconciled if it develops, as seems probable, that photosynthesis is carried on in at least two stages, one of which is physical and one of which is chemical. Brown and Heise thus may have been measuring one step in the process and Matthaei another.

Atmospheric Pressure.—Friedl (1902) showed that variations in the atmospheric pressure exert a marked influence upon photosynthesis. When the pressure is high, photosynthesis is increased.

Oxygen.—Since photosynthesis is a function of green plants, which require free oxygen for their normal life processes, one would expect oxygen to be necessary for photosynthesis. Briggs (1920) found this to be the case, but Harvey (1928) reports that marine algæ can carry on photosynthesis when illuminated even in the complete absence of oxygen. This matter should be studied further.

Leaf Structure.—Some leaves contain more stomata than others and, although in most leaves the stomata are spaced so as to permit of optimum diffusion (Chap. XVIII), leaves with stomata on both sides are especially favored in this respect. If the stomata are plugged by smoke and dust, inward diffusion of carbon dioxide will be hindered. One of the chief sources of harm of smelter smoke and dust is this plugging of the stomata, which more than counteracts the beneficial effect of the increase of carbon dioxide in the atmosphere of the vicinity. House plants are benefited by an occasional washing of the leaves, which removes all collected dust particles; and one of the benefits of a rain aside from increasing the water supply is this cleansing effect upon the vegetation.

Amount of Water.—Photosynthesis depends primarily upon water, since in the manufacture of food the carbon of the carbon dioxide is united to the water brought up from the soil; but, in accordance with the law of limiting factors, increasing the water supply of the leaf will increase the photosynthesis only up to a certain point. Wilting leaves not only contain too little water to photosynthesize efficiently but the wilting results in closed stomata thus prohibiting the entrance of carbon dioxide. In the cowslip (*Caltha*) and *Hydrangea*, however, the stomata remain open even in the wilted condition and the leaves are still able to make some starch.

Soil Salts.—An excess of salts in the soil retards photosynthesis probably due to its success in checking the intake of water as will be explained in Chapter XIX. Halophytes (plants living in salty or brackish water) grow, though slowly, even in salty soils because their stomata do not close like those of normal plants and the entrance of carbon dioxide is not prohibited.

Chlorophyll.—Not only is chlorophyll absolutely essential for photosynthesis, but the amount of food manufactured by green plants varies almost directly with the amount of chlorophyll present (Emerson, 1929). Grapes have larger fruits when many leaves are present, and there is a definite correlation between the amount of chlorophyll and the dry weight of tops in corn (Sprague and Shive, 1929). The effect of hail on a corn crop, the damage done by locusts, and the results of defoliation (either accidental or experimental) all attest the importance of the quantitative relation between manufactured food and chlorophyll. As one passes from a country where there is plenty of chlorophyll to a desert region where this substance is much rarer, one is much impressed by the fact that lower animals, the human inhabitants, and even the cities all grow progressively rarer, paralleling the decrease in chlorophyll.

Among the factors necessary for the formation of chlorophyll, light is of the highest importance. Dangeard claims to have grown the alga, *Scenedesmus*, in the dark for eight years, at the end of which time the plant was as green as ever, with chlorophyll that showed the normal spectrum. This is rather astonishing if true, but practically all angiosperm leaves are yellow in the dark, as anyone who has ever turned over a board which has been lying upon the grass for some time may attest. Such yellow leaves (said to be *etiolated*) soon become green when exposed to the light. Even a weak light is sufficient and is, in fact, better than very strong light. Wiesner (1874) explained this by showing that strong light decomposes chlorophyll. The two processes of formation and decomposition go on simultaneously, and in strong light the decomposition is more rapid than the formation, while in weak light the reverse is the case, the decomposition being nearly absent. Attention has previously been called to the fact that shade plants are greener than sun plants and we have here the explanation. Some plant organs such as pine seedlings and young fern fronds may become green even in the dark (Lubimenko, 1910) but this formation in the dark is less strong and the green material formed in these cases may not actually be chlorophyll.

Wiesner investigated the effect of light quality upon chlorophyll formation, using the same method which Sachs had used for determining which wave lengths were best for photosynthesis. In weak light the greening is more rapid in red light, but in strong light the plants green more rapidly in the blue end of the spectrum. This has been explained by assuming that red light is more effective both in the formation and destruction of chlorophyll. In the weak light the destructive action does not appear, but in strong light this action is very pronounced so that the plants under the blue become visibly green more rapidly. Another explanation is that the strong red light has an injurious effect upon some chemical reaction which precedes the chlorophyll formation. It is only the visible portions of the spectrum which produce greening; neither the infra-red heat waves nor the ultra-violet cause an appreciable increase in chlorophyll. Sayre, using more refined methods, determined the limits as 300 and 680 mμ, at which regions chlorophyll formation stopped rather abruptly.

Chlorophyll production also depends upon the temperature with a minimum at 2–4°, an optimum about 30°, and the maximum at 40°. The optimum for chlorophyll formation is thus seen to be about the same as for photosynthesis.

Certain mineral salts especially those of magnesium and iron are necessary for chlorophyll formation. In the absence of either of these elements the plants are yellowish-white or *chlorotic*. Only a trace of iron and magnesium are sufficient to produce greening. It was formerly thought that iron entered into the composition of chlorophyll; now, however, it is known that the chlorophyll molecule contains magnesium but not iron. According to Fernald, plants grown in a soil rich in magnesium are of a richer green than those grown in ordinary soils, and Zaitseva (1928–1929) experimentally increased the chlorophyll yield of plants by suitable additions of magnesium to the nutrient medium.

Oxygen is needed for greening. Etiolated plants in a chamber with no or little oxygen present will remain etiolated even in the light.

Palladin (1891) has shown that carbohydrates must be present before greening occurs. If an etiolated leaf of wheat and one of a bean are removed and floated on water in the light, the former will become green much more quickly than the latter. This is associated with the higher carbohydrate content of wheat leaves. If the bean leaf is floated on a 5–10% sugar solution it also will become green.

As a final prerequisite for chlorophyll formation, must be mentioned the presence of *chlorophyllogen* (also called *protochlorophyll*), a pigment which is formed antecedent to chlorophyll and which gives rise to it. Monteverde and Lubimenko (1911) have proposed that chlorophyllogen arises from *leucophyll,* a colorless chromogen. The chlorophyllogen is a very unstable material very much like chlorophyll, but it arises from the leucophyll quite independently of light. The change from chlorophyllogen to chlorophyll, on the other hand, depends upon light. The green material found in the inner seed coats of melon and lemon seeds and in conifer seedlings grown in the dark is probably chlorophyllogen rather than chlorophyll, although it is possible that in exceptional cases chemical activity might replace the transformation ordinarily effected only by the light. With the exception of light, none of the other conditions mentioned above such as a favorable temperature, presence of carbohydrates, or oxygen is necessary for transforming chlorophyllogen into chlorophyll. Hence these necessary conditions for greening must be prerequisites for the transformation of the leucophyll into chlorophyllogen or for the formation of the leucophyll.

For the formation of the *plastids,* as has been shown by Lindstrom (1918), certain hereditary factors must be present. Likewise, in the virescent forms of kafir and in variegated forms of *Pelargonium* and *Hydrangea,* the lack of chloroplasts has been shown to be due to a genetic factor.

Amount of Carbon Dioxide.—Of all the factors necessary for photosynthesis, the amount of carbon dioxide is generally the *limiting factor.* The temperature is high enough and there are enough mineral salts, oxygen, etc., present to produce the maximum amount of carbohydrates with the carbon dioxide which is available. This means that at ordinary temperatures of the growing season and on a sunny day, the amount of food manufactured could not be increased by raising the temperature or increasing the illumination but only by increasing the amount of carbon dioxide in the air. The 0.03% of carbon dioxide in the normal air is far below the optimum. The amount of carbon dioxide may be increased up to 10% with benefit to the plant. As the amount is increased to 1%, there is a very rapid rise in photosynthetic activity, according to Godlewsky. From this point on to 10%, there is a slow rise, which requires increased temperature and illumination to utilize, but 10% is the maximum, and if the amount of carbon dioxide is increased beyond this figure there is a decrease in activity. An example of the many experiments carried on in recent years on "fertilization" with carbon dioxide is the work of Lundegardh, who burned alcohol lamps in a specially prepared glass house, keeping the carbon dioxide in the air at 3–4 times the normal amount. Cucumbers grown under these conditions yielded 30–45% more than the controls, and strawberries not only ripened earlier but had about 12% more fruit. This experimentation can be done practically, of course, only in greenhouses, where diffusion can be checked, although Riedel near Essen, Germany, carried on some experiments in the field. The effect of carbon dioxide on plant production is an extremely important matter although, for the most part, it seems to be almost entirely beyond human power to increase or diminish the amount normally present. If carbon dioxide were more plentiful, food would be even more abundant and cheaper.

Daily Course of Photosynthesis.—With the present discussion of limiting factors in mind, it is not difficult to picture the average daily course of photosynthesis. Before daybreak, light is the limiting factor, but some time before full daylight, the light has ceased to be a limiting factor and photosynthesis is proceeding at full speed, with carbon dioxide probably as the limiting factor. Towards the middle of the day, a lack of water may become the limiting factor, in which case the stomata may close somewhat and the incoming carbon dioxide will be proportionately checked. In the middle of the afternoon, photosynthesis generally slows up, not because of *external* limiting factors but because of an *internal* one, viz., the accumulation of starch in the leaves which hinders more from being made. This drop in the photosynthetic curve is later followed by a sharp fall (after dusk), owing to the lack of light; and during the night, even in full moonlight, photosynthesis is nil for all practical purposes. However, if the curve of photosynthesis were drawn to show the action during successive minutes, it would be extremely irregular. Maximov (1928) has shown that photosynthesis may vary 25–100% in successive minutes possibly because of the great variations in stomatal movement noted by Agamaov (1927). Kostychev has also noted that even with plenty of light and carbon dioxide, the plant may suddenly stop manufacturing food for a short time and then resume operations again. This brief cessation, like the drop in the afternoon, may be associated with some internal factor connected with the photosynthetic machinery. Because smoke does not continuously pour out of the chimney, however, is not an absolute proof that the factory has shut down or stopped operations.

Summary.—If one wished to make a corn plant produce the maximum amount of carbohydrate in a given period of time, the following favorable conditions would have to be provided: (1) See that the plants maintain a healthy rich green color. (2) Provide abundant water. (3) Artificially maintain a temperature of about 35° C. (4) Illuminate the plant continuously with light of the optimum intensity. (5) Increase the carbon dioxide content of the air to at least 1%. Ordinarily both light and temperature are below the optimum but with a limited amount of carbon dioxide at the disposal of the plant, light and temperature are not limiting factors.

QUESTIONS

1. Are there any plants which lack leaves? If so, how do they manage to live?
2. Why do potato beetles affect the size of the crop?
3. Is carbon dioxide more soluble in warm or cold water? What bearing does this have upon life in Arctic regions?
4. Parasitic plants are not green. Explain.
5. How are celery plants "bleached"? Explain.
6. Why are plants commonly placed in an aquarium?
7. Frequently the lower leaves of a plant have longer petioles than the upper ones. Why?
8. Which are generally bigger, opposite or alternate leaves? Why?
9. Can a lawn be maintained best under elms or maples? Why?
10. In what part of a tree are the leaves borne? Why?
11. Is it ever advantageous to a plant to be heliophobous during a part of its life and heliophilous during another? Illustrate.
12. When do wild flowers on the deciduous forest floor usually blossom? Why?
13. In which month do plants grow fastest? Why?
14. On the Newfoundland Banks the catch of fish is said to be bigger in sunny seasons than in cloudy ones. Why?
15. Who first showed that the carbon of plants comes from the air rather than from the soil?

16. What is chlorosis? Why is it objectionable? How may it be remedied? How does it differ from albinism?

17. Discuss Liebig's law of the minimum with special reference to photosynthesis.

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CHAPTER V

CHEMISTRY OF CHLOROPHYLL: THE CAROTINOIDS

> I will allow there is much to be said for yellow and green.
> —EUGENE FIELD.

Plastids.—The chlorophyll in most plants, as can be easily seen by examining a leaf under the microscope, is not distributed evenly through the protoplasm but is contained in special structures called *plastids*. These green bodies which contain the chlorophyll are of various shapes and sizes. In many of the algæ they are ribbon-(*Spirogyra*), star-(*Zygnema*), or cup-(*Chlamydomonas*) shaped, but in the higher plants the ordinary shape is that of an elongated hollow spheroid with a central vacuole. As the cytoplasm moves about the cell, it may carry with it the chloroplastids, which in this case serve as an index to the movement.

Origin of Plastids.—The plastids do not originate *de novo*, i. e., out of nonplastid material, but come from preëxisting plastids or their forerunners. In some cases they have been seen to arise from the division of plastids and, as a result of these observations, it has been proposed that they originated in the cells of plants as independent one-celled organisms which have been held there and imprisoned. The work of Lewitski (1910), Zirkle (1926), and others, however, has led to the conclusion that the plastids are not imprisoned algæ but that they arise from minute bodies in the protoplasm not seen by ordinary methods of staining which belong to the class of bodies called *chondriosomes*. These structures are essential parts of the protoplasm which by their division and increase in size may give rise to the plastids.

Relation of Plastid and Pigment.—The plastid seems to be a spongelike matrix of protoplasm in the meshes of which is the chlorophyll. The condition of the pigment within the plastid has been the subject of much dispute. Some workers have expressed the view that it is distributed throughout the groundwork of the plastid as discrete colloidal particles; but most of the evidence indicates that it is dissolved in lipoids (Chap. XIII) in the plastids. The followers of both theories assume that the chlorophyll is in a colloidal state; but the former hypothesis would give more the impression of a suspension, while the latter seems to convey the idea of an emulsion. The fluorescence so characteristic of chlorophyll is seen only when in solution; and when chlorophyll is shaken with a lipoid substance (oleic acid, lecithin, cholesterin) a marked fluorescence occurs, resembling that seen in the living plant. Also the absorption bands of chlorophyll in the cell are very similar to those of chlorophyll in lecithin.

Priestley and Irving (1907) have found the chlorophyll distributed only at the surface of the plastid where it can absorb as much light and expose as much surface as possible. Here it seems to be in a very thin layer, adsorbed to the protein of the plastid. Zirkle (1926), however, finds the chlorophyll evenly distributed throughout the protein ground-substance of the plastid. The conditions necessary for the formation of the chlorophyll in the plastid have been stated in the previous chapter.

Chlorophyll.—Chlorophyll (Gr., green leaf) which received its name from Caventou and Pelletier (1819) was not isolated until nearly a century later (1912) when Willstätter, as a result of experiments already classic, obtained pure chlorophyll and determined its chemical composition. To him and his students (especially Stoll) most of our knowledge of chlorophyll is due.

Willstätter showed that chlorophyll as ordinarily obtained is a mixture of two substances which he has called chlorophyll "A" and chlorophyll "B." The former has the formula $C_{55}H_{72}O_5N_4Mg$ and the latter $C_{55}H_{70}O_6N_4Mg$. While some plants are richer in chlorophyll than others, the average total yield from 1 kg. of fresh leaves is 1–2 g. and of dried leaves 5–10 g. The proportions of *A* to *B* are as 31 to 11 in land plants; in the brown algæ, 16 to 1; and in the green algæ, 31 to 22. Both these chlorophylls are amorphous in the plant but when extracted with ethyl alcohol they form green crystals. They have different solubilities in organic solvents, however, and are separated from each other because of this property. Chlorophyll *A* is blue-green in transmitted light and blood-red by reflected light, while chlorophyll *B* is yellow-green by transmitted light and brownish-red by reflected light. Both show this peculiar property of *fluorescence.*

Chlorophyll is an *ester,* that is, it is a combination of an acid with an alcohol. If an alcohol with the formula ROH is mixed

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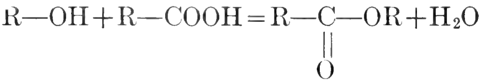
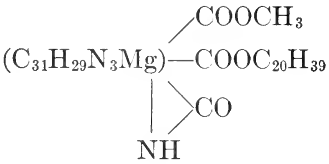
with an acid R—COOH, water is split off and the resulting product is an ester.

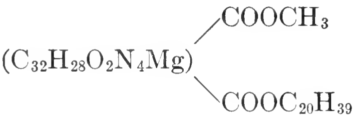


$$R{-}OH + R{-}COOH = R{-}\underset{\overset{\|}{O}}{C}{-}OR + H_2O$$

Chlorophyll *A* has the structural formula

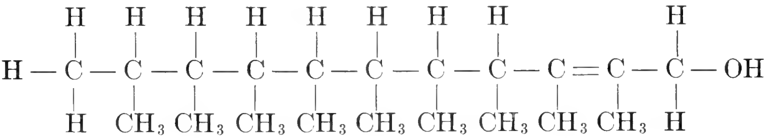


and *B*



They are both thus seen to be esters of methyl alcohol (CH_3OH) and another alcohol ($C_{20}H_{39}OH$) called phytyl alcohol. Chlorophyll *A* in addition contains a ring formation known as a *lactam* ring.

Phytol.—The phytyl alcohol or *phytol,* which makes up a third of the chlorophyll molecule, is thought to have the following construction:

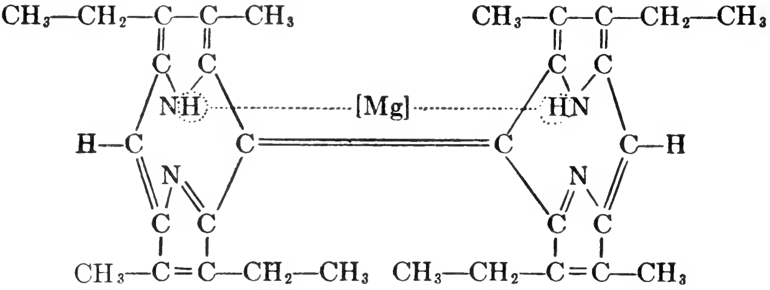


$$H - \underset{H}{\overset{H}{C}} - \underset{CH_3}{\overset{H}{C}} - \underset{CH_3}{\overset{H}{C}} - \underset{CH_3}{\overset{H}{C}} - \underset{CH_3}{\overset{H}{C}} - \underset{CH_3}{\overset{H}{C}} - \underset{CH_3}{\overset{H}{C}} - \underset{CH_3}{\overset{H}{C}} - \underset{CH_3}{C} = \underset{CH_3}{C} - \underset{H}{\overset{H}{C}} - OH$$

It is a colorless, oily liquid which is found nowhere else in nature. When the leaves are treated with ethyl alcohol in the ordinary methods of extracting chlorophyll from leaves, the phytyl alcohol is replaced by the ethyl so that what one obtains is *ethyl chlorophyllide.* Methyl alcohol may be used in the same way. These chlorophyllides are crystalline while the original phytyl chlorophyllide or chlorophyll is amorphous.

The Acid.—The acid in the ester has been called *chlorophyllin.* It is a tri- or di-carboxylic acid, as shown by the formula, in which only two of the acid groups are effective. One of these is joined to a methyl alcohol and the other to the phytol. The third or ineffective one is in the lactam ring of chlorophyll *A*, while in chlorophyll *B* it is absent entirely.

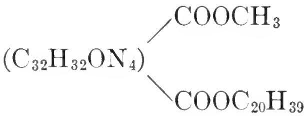
When chlorophyll is heated with alkalies at a high temperature, the carboxyl groups are broken off leaving a substance called *etiophyllin* ($C_{31}H_{34}N_4Mg$) with the following proposed structural formula (Fischer and Klarer):



Here we see that magnesium is the heart of the etiophyllin molecule and that in this acid part of chlorophyll, Mg plays the central rôle. No iron or other metals enter at all into the chlorophyll molecule.

Two Series of Decomposition Products.—The preceding paragraph has shown what happens when chlorophyll is treated with alkalies. The carboxyl groups are driven off successively giving products known as *phyllins,* which contain magnesium. The green color of the chlorophyll, as previously hinted, is connected with the presence of the magnesium, and it is not surprising that these decomposition products retain their green color. The final phyllin, when all the carboxyl groups have been driven off, is the etiophyllin mentioned above.

If chlorophyll, on the other hand, is treated with acids, the magnesium is replaced by hydrogen and a series of products results which are called *phytins.* Thus chlorophylls *A* and *B* give *phæophytins A* and *B*, the former of which has the formula:

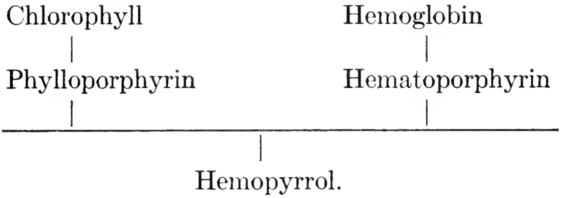


$$(C_{32}H_{32}ON_4)\left\langle\begin{array}{l}COOCH_3\\ COOC_{20}H_{39}\end{array}\right.$$

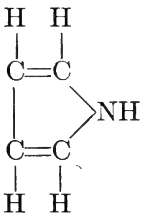
If a phyllin is treated with an acid one gets magnesium-free substances to which have been given the name of *porphyrins.*

Chlorophyll, Hemoglobin, and Evolution.—From an evolutionary point of view it has been highly suggestive to observe that there is a striking chemical similarity between chlorophyll, the characterizing green pigment of plants, and hemoglobin, the characteristic red-colored material of the blood of animals. If chlorophyll is treated with an acid and then an alkali, the porphyrin resulting is called *phylloporphyrin;* and if hemoglobin is treated in a similar manner, the substance *hematoporphyrin* is obtained. These two porphyrins have almost identical spectra and formulæ. The iron has been removed from the hemoglobin in the same way that the magnesium was removed from the chlorophyll.

A further connection between chlorophyll and hemoglobin is shown by the fact that these two porphyrins give the same decomposition products, as shown in the accompanying diagram:

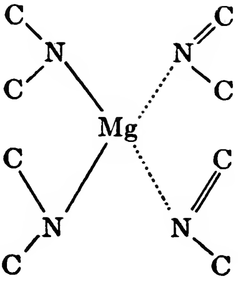


Nentski and Marchlewski (1901) obtained hemopyrrol from both of them. The pyrrol ring is seen from the formula given on page 48 to be found in four different places in etiophyllin. Iron has been found by Baly to be one of the best catalyzers for the formation of the pyrrol ring,

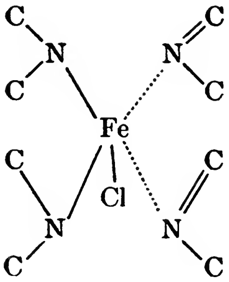


and this may explain its importance in chlorophyll formation.

If the formulæ for chlorophyllin and hematin are compared, it will be seen that the chief difference is the element at the center of the molecule. In chlorophyllin it is magnesium while in hematin it is iron. In agreement with this are the results of Küster (1926), who showed that both hemoglobin and chlorophyll can be derived from koporphyrin of colorless organisms such as yeasts. Hemoglobin is derived by eliminating two molecules of formic acid and adding iron to the pyrrol groups, while chlorophyll is derived by eliminating a molecule of carbon dioxide and adding magnesium. Similarly, by removing the magnesium from protochlorophyll, Noack obtained protophæophytin, which has a brilliant red color and is related to the bilipurpurin of animals. Also when magnesium is added to bilipurpurin (also called phylloerythrin), a substance is obtained with almost the same spectrum as chlorophyll.



chlorophyllin



hematin chloride

In addition to the similarity in chemical analysis between the hemoglobin and chlorophyll, their physiological resemblances are equally striking. Both are pigments; according to Palladin both may be concerned in the transfer of oxygen; Manoilov (1922) has produced evidence that the chemical tests which distinguish male from female blood may also be applied to male and female chlorophyll in diœcious plants; and Raber (1930) called attention to the fact that liver extract, a common treatment for pernicious anæmia, also checked somewhat the etiolation of green plants when placed in the dark.

These results obtained by biochemistry throw an extremely interesting light on the evolutionary development of plants and animals at the time when these two branches of the organic world were commencing to diverge from each other. Differences between organisms are to be found not only in structure and function, but associated with these differences are differences in metabolism, which in the one group of organisms (plants) have produced independent individuals able to manufacture their own food, and in the other group (animals) a type of organism which must live dependently upon food built up by the former group.

The Carotinoids.—Associated with the chlorophyll in the leaf are two other pigments, which are extracted by ethyl alcohol at the same time as the chlorophyll and later must be separated from it if pure chlorophyll is desired. The most important of these is *carotin* which receives its name from its color (Gr., reddish-yellow) and its abundance in carrots. The color of yellow and orange petals is commonly due to this pigment. It is also abundant in yellow tomatoes and in seeds such as yellow corn.

Carotin is very soluble in carbon disulphide, from which it may be precipitated as orange crystals, which are orange-red by transmitted light and greenish-blue by reflected light. Willstätter has determined the formula as $C_{40}H_{56}$. *Lycopin*, isolated from red tomatoes and red peppers, is an isomer. If tomatoes which are normally red are ripened at temperatures above 30° C. they will be yellow instead of red, showing the presence of the isomer, carotin.

Closely associated with carotin and related to it is *xanthophyll*, $C_{40}H_{56}O_2$. This seems to be an oxide of carotin, but it has not yet been possible to oxidize carotin to xanthophyll in the laboratory. This does not preclude the possibility, however, that the two substances may be transformed into each other in the plant. Such transformations might then be analogous to the changes from hemoglobin to oxyhemoglobin, and the reverse, in the blood of animals during respiration.

Xanthophyll is more yellow than carotin, is much less soluble in carbon disulphide, and more soluble in acetone; and, as the two chlorophylls are separated from each other on the basis of their differential solubilities in various solvents, in like manner the carotinoids may be separated from each other and from the chlorophylls.

From the brown algæ Willstätter has isolated a pigment called *fucoxanthin* with the formula $C_{40}H_{54}O_6$ which indicates its relation to the other carotinoids. It is brownish-red with basic properties.

The carotinoids, unlike chlorophyll, will form in darkness and seem to undergo very little change or decomposition in the digestive tract of animals. The yellow yolk of eggs contains carotinoids, and if chickens are fed on white corn instead of yellow and kept from getting green foods, the yolks soon become pale and colorless. Similarly the yellow color of the feathers and of the leg scales is connected with an abundant carotinoid diet.

QUESTIONS

1. What are mitochondria and chondriosomes?
2. How can the chlorophylls be separated from the carotinoids?
3. How are the chlorophylls separated from each other?
4. How is xanthophyll separated from carotin? Name specific solvents in each case.
5. What are esters? Give examples.
6. Plants grown on serpentine soil seem to be especially rich in chlorophyll. Explain.
7. One sees in newspapers many advertisements of iron as a cure for anæmia. Is there any basis for such advertising?
8. Why do autumn leaves often turn yellow?
9. In the winter time more artificial butter coloring is needed than in the summer. Why?
10. Why is butter yellow instead of green?
11. What seems the most logical source of the blood materials in animal metabolism?

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CHAPTER VI

THE PHOTOSYNTHETIC PROCESS

Such was the process.
—OTHELLO 1:3.

The Formaldehyde Hypothesis.—It has been previously shown that the plant which contains chlorophyll is able to make carbohydrates out of the raw materials of carbon dioxide and water. Also for every molecule of carbon dioxide taken in from the air a molecule of oxygen is given off. The sum total of reactions can then be expressed as follows, if we assume that a sugar like glucose is the first product of photosynthetic activity:

$$6CO_2+6H_2O=C_6H_{12}O_6+6O_2.$$

Such an equation tells us only the alpha and the omega. It states that water and carbon dioxide are used and that glucose is formed. It also shows that for every molecule of carbon dioxide used a molecule of oxygen is released. But all that such equations state are simply the substances and the proportions; they tell nothing whatever of the intermediary steps or of the numerous reactions which may intervene between the beginning and the end.

In order to throw some light on these dark places von Baeyer (1870) proposed that formaldehyde was an intervening product. To explain what actually took place within the leaf the following reactions were assumed:

$$\begin{aligned}&CO_2 \rightarrow CO+O\\&H_2O \rightarrow H_2+O\\&CO+H_2=CH_2O\\&6CH_2O \rightarrow C_6H_{12}O_6.\end{aligned}$$

These equations mean that the carbon dioxide is broken up into carbon monoxide and oxygen, while the water is broken into hydrogen and oxygen at the same time. The carbon monoxide and hydrogen then unite to form formaldehyde, six molecules of which are condensed to form a molecule of glucose. There are thus seen to be two distinct reactions, (1) the formation of the formaldehyde and (2) its polymerization into the sugar, of which only the first may be dependent upon light.

Among the serious objections to this theory are that carbon monoxide is never found in the free state in the plant (except in a few cases as a product of respiration) and that it is a very poisonous anæsthetic. According to Kraschennikov and Sulander, no green plants are able to use carbon monoxide and 0.5% was sufficient to inhibit the growth of lupine seedlings. Although Bottomley and Jackson (1903) have reported that if carbon monoxide is substituted for carbon dioxide *Tropæolum* forms starch and grows well, the majority of evidence points the other way.

Erlenmeyer (1877) tried to modify von Baeyer's theory to make it acceptable, and suggested that the carbonic acid is reduced to formic acid and oxygen, after which the formic acid is then further reduced to formaldehyde and oxygen, or that the reducing action may give rise directly to formaldehyde and oxygen:

$$CO_2+H_2O=H_2CO_3$$
$$H_2CO_3=H_2CO_2+O$$
$$H_2CO_2=CH_2O+O.$$

According to the first view, both formaldehyde and formic acid are produced while, according to the second, only formaldehyde. Formic acid is also poisonous to the plant, but the fact that it is never present in large quantities, being reduced (as soon as formed) to formaldehyde (which in turn is immediately polymerized), might answer this objection.

Bach, who was the first to attempt to produce formaldehyde *in vitro* from carbon dioxide and water, proposed that carbonic acid was reduced to a peroxide and formaldehyde, after which the peroxide was also reduced. This hypothesis, in a somewhat modified form, is held today by many workers, who consider that the reactions occur in the following steps:

$$CO_2+H_2O=H_2CO_3$$
$$H_2CO_3+H_2O=HCOOH(\text{formic acid})+H_2O_2$$
$$HCOOH+H_2O=CH_2O(\text{formaldehyde})+H_2O_2$$
$$2H_2O_2=2H_2O+O_2.$$

This last step is accomplished through the aid of the enzyme, catalase.

All of the above theories have assumed the production of formaldehyde as a step in the photosynthetic process. Although poisonous, it is quickly polymerized and is never present in large quantities. Furthermore, many researches have shown that it can be used by the green plant. Grafe (1911) showed that green seedlings grown in an atmosphere containing formaldehyde without carbon dioxide increased in weight when grown in the light, as compared with similar plants grown in the absence of formaldehyde. This has also been supported by Miss Baker (1913). But it should be added that all the experiments carried on to show that plants can use formaldehyde are valueless as far as the question at hand is concerned if carried on in the light. Under these conditions the formaldehyde may be first oxidized to formic or carbamic acid and then used by the plant in the normal manner. Such experiments to be of value must be performed in the dark, and have been, in fact, by Jacobi (1919), Sabalitschka (1924), Bodnar (1927), and others, all of whom agree that the plant does increase in dry weight under these conditions.

It has further been demonstrated that plant cells containing chlorophyll will give an aldehyde test if exposed to light and carbon dioxide while the cells of fungi do not. Formaldehyde has been commonly found in distillate from green leaves, but it has also been found in distilled hay, where there is no photosynthesis. It is not certain from these experiments, then, whether the aldehyde is produced in photosynthesis or whether it is a decomposition product.

Bach had demonstrated that formaldehyde could be produced *in vitro* from carbon dioxide and water in the presence of sunlight provided that some sensitizer or catalyzer was present, but this entire question as to whether or not photosynthesis was a vital process was reopened by Usher and Priestley (1906–1911), who removed chlorophyll from the leaf, spread it out in thin films of gelatin, and exposed it to the light. A formaldehyde test was easily obtained at the end of an hour, but at the same time the chlorophyll was decolorized indicating decomposition, which would indicate that the aldehyde found was not formed from a photosynthetic action but as a decomposition product of the chlorophyll. If, however, catalase obtained from the liver of a sheep was added to the gelatin-chlorophyll film, the chlorophyll was still green at the end of the day. They explained this by assuming that one of the products formed is hydrogen peroxide which causes the bleaching of the chlorophyll. If catalase is present, which is the case in the cells of the plant, the peroxide is broken down into water and oxygen and the chlorophyll is not decomposed. An excess of formaldehyde may poison the catalase so that the peroxide is not broken down, in which case the peroxide bleaches or decomposes the chlorophyll.

Schryver (1910) and others have criticized and supplemented the work of Usher and Priestley in some particulars. Schryver found that small amounts of formaldehyde were produced in the sunlight in an atmosphere free from carbon dioxide, which would indicate that at least some of the formaldehyde produced is not related to photosynthesis. This may, however, be a part of the mechanism to regulate the amount of aldehyde present. If more is produced at any one time than can be condensed into glucose, it unites with the chlorophyll and is later freed at a time when it can be converted into sugar.

The work of Klein and Werner (1926) was construed for some time as giving decided support to the formaldehyde hypothesis. Using the very sensitive dimedon test for aldehyde, they seemed to have found that formaldehyde was actually formed in the cell during photosynthesis; but Barton-Wright and Pratt (1930) showed that this was due to the action of light on bicarbonates and carbon dioxide and was independent of the photosynthetic mechanism.

In summing up our present knowledge of this question, all we can say is that the formaldehyde theory has not been proved. But neither has it been disproved nor has any very serious objection been raised to it. Although toxic, as stated above, it is probably polymerized to sugars as soon as formed, so that little is ever present in the cell. This hypothesis agrees best with the known facts of photosynthesis and is, therefore, becoming more firmly entrenched as the years go by.

Further Steps in the Process.—After the chemistry of chlorophyll and the carotinoids had been worked out, Ewart (1918) and many others tried to make use of these formulæ to construct equations which might show more fully what goes on in photosynthesis. As a sample of the less fantastic of these "arm-chair" chemical equations are presented those of Gordon (1929), who has proposed the following scheme:

$$\underset{\text{chlorophyll B}}{6C_{55}H_{70}O_6N_4Mg} + 6H_2O = \underset{\text{chlorophyll A}}{6C_{55}H_{72}O_5N_4Mg} + 6O_2$$

$$\underset{\text{chlorophyll A}}{6C_{55}H_{72}O_5N_4Mg} + 6CO_2 = \underset{\text{chlorophyll B}}{6C_{55}H_{70}O_6N_4Mg} + \underset{\text{sugar}}{C_6H_{12}O_6}.$$

Spoehr and McGee (1924) have suggested that the carbon dioxide unites with protein in the leaf as carbon dioxide in the blood unites with hemoglobin. From this protein compound the carbohydrates are split off without the intermediate steps of formaldehyde or carbonic acid. This is one of the few modern theories which does not presuppose the formation of formaldehyde and, although interesting, is not so promising as some others.

Hypothesis of Willstätter and Stoll.—These men, who did so much to clarify the chemistry of chlorophyll, also tried to explain (with not so much success, however) the function of chlorophyll. According to them, photosynthesis takes place in four successive steps:

1. The first step is the union between the carbon dioxide, water, and chlorophyll to form a chlorophyll-carbonic acid compound. This is a chemical process and as such does not require light. It, consequently, has a temperature coefficient of 2 or more.

2. In the next step, the energy absorbed from the sunlight causes a rearrangement of the molecules in this compound so that some sort of organic peroxide is formed. This is the *photochemical* stage of the process for which light is necessary and which has a temperature coefficient of 1.

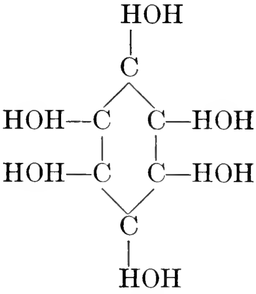
3. From this organic peroxide, formaldehyde is split off by means of enzymes, thus forming formaldehyde, chorophyll, and oxygen. It also is a chemical reaction with a temperature coefficient of at least 2. As an argument in favor of the formation of formaldehyde, they called attention to the fact that according to the ordinary chemical equation, $CO_2+H_2O=CH_2O+O_2$, the $CO_2:O_2$ ratio is 1, while if formic acid were formed ($2CO_2+2H_2O=2HCOOH+O_2$), the ratio would be 2. But Kostychev has pointed out that if sugars are the final product, they can be

made from formic acid or formaldehyde regardless of the $CO_2:O_2$ ratio in these equations. The fact that the ratio happens in nature to be 1 proves only that carbohydrates are the chief end products and not that formaldehyde is an intermediary step. That an enzymatic factor occurs somewhere in the process is indicated also by the work of Molisch (1925), who showed that leaves killed by drying or freezing retain somewhat their power of photosynthesis, while leaves killed by boiling or ether do not.

4. The formaldehyde is polymerized to sugar.

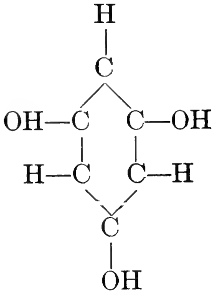
The third step in this scheme is sometimes known as the "Blackman reaction," because according to Blackman this is the step which, under ordinary light conditions, controls the rate of photosynthesis. Thus Warburg has found that KCN affects this stage of the process, but Emerson and Arnold (1932) have produced evidence that this reaction is not affected by narcotics. They also have concluded that the light reaction takes place in about 1/100,000 of a second, while the dark (Blackman) reaction requires 0.04 sec. at 25° C. and about 0.4 sec. at 1.1° C. It is thus easy to see that various limiting factors may limit different stages in the process. Carbon dioxide may seriously limit the first stage, light the second, and temperature the third.

Baly's Work.—Baly found that when a solution of formaldehyde was placed in a quartz tube and exposed to rays of high frequency, at the end of the experiment about 34% of the material had combined to form sugars of various sorts (10% glucose and about 10% phloroglucin and inosite) and various impurities (14%). He believes that the active formaldehyde forms a ring compound when it first condenses:



and that from this ring four things may happen:

1. Water may split off producing the phloroglucin:



2. The ring may split forming a chain compound,—the glucose.
3. The same thing may happen forming fructose.
4. The compound may "settle down" and become inactive forming inosite. The plant makes all of these at once but *in vitro* no fructose is found because it is very unstable and is destroyed as fast as formed. Furthermore when a solution of carbon dioxide was exposed to ultra-violet light, formaldehyde was formed and then sugars.

In these experiments, catalysts of various sorts are included in the tubes to hasten the chemical reactions, and it was Baly's experience that the color of the catalyst was important. Thus when the catalyst had a green surface ($NiCO_3$), there were better results than when a white catalyst was used; but pink ($CoCO_3$) was as efficient as green. It is his belief that chlorophyll A combines with carbon dioxide to form chlorophyll B and formaldehyde. The chlorophyll B then reacts with carotin and water to form A and xanthophyll, which in turn is then reduced by some enzyme to re-form the carotin and release oxygen, after which the formaldehyde is polymerized to form a hexose sugar.

$$\underset{\text{(chlor. A)}}{C_{55}H_{72}O_5N_4Mg}+CO_2=\underset{\text{(chlor. B)}}{C_{55}H_{70}O_6N_4Mg}+CH_2O$$

$$C_{55}H_{70}O_6N_4Mg+\underset{\text{(carotin)}}{C_{40}H_{56}}+H_2O=C_{55}H_{72}O_5N_4Mg+\underset{\text{(xanthophyll)}}{C_{40}H_{56}O_2}$$

$$C_{40}H_{56}O_2\rightarrow C_{40}H_{56}+O_2.$$

This scheme has the further advantages of being simple and of explaining the presence of all the materials found in close association with chlorophyll. The chief objection to all the work of Baly is that it has been done entirely *in vitro*, and, as Barton-Wright has phrased the matter, "it is somewhat absurd to compare the rough-and-tumble heroics of Baly's test tube experiments with the marked smoothness of the processes of the photosynthetic mechanism of the plant."

Part Played by Light.—Many investigators have attempted the formation of carbohydrates *in vitro*, replacing light by some other form of electrical or chemical energy. Butlerow (1861), using limewater as a catalyzer, obtained from trioxymethylene, which is a polymer of formaldehyde, a syrup with a bitter-sweetish taste. Loew in a similar manner produced from formalin a colorless syrup, which he called formose, that will reduce Fehling's solution. Löb, using a silent discharge as a source of energy, found that carbon dioxide and water produced formaldehyde and in addition carbon monoxide and hydrogen peroxide. These last two products are especially interesting in the light of the work of von Baeyer, Usher, and Priestley. The latter investigators found that carbon dioxide and water, when placed in a quartz tube along with an alkali and subjected to the rays of a mercury vapor light especially rich in the violet and ultra-violet rays, produce formaldehyde and even carbohydrates; but Stoklasa found that for carbohydrate production nascent hydrogen must also be present. The most important work in this field is that of Baly, described above.

Experiments such as these have led many to believe that the light energy is in some way transformed into electrical energy; but the boundary line between these two phases of energy seems in certain regions of the spectrum to be especially indistinct. It is a matter of common knowledge that light is a form of energy, that it can be transformed into other forms, and that the plant is able to transform this kinetic light energy into the potential energy of carbon compounds. Light is able to decompose silver salts, fade rugs, etc., and its decomposition or rearrangement of the carbon dioxide molecule is no more strange.

Part Played by Chlorophyll.—It was once thought that chlorophyll was an absorbing screen for gathering the rays of the orange-red region which are most used in photosynthesis. Later it was considered by some as a sort of sensitizer or catalyzer which permitted the protoplasm of the chloroplastid to carry on the work of photosynthesis. Bacteria when impregnated with eosin, a red dye, are very sensitive to light and soon succumb, while in the dark they are not affected; and in some such way, it was proposed that chlorophyll affected the sensitiveness of the plastids which contained it.

The phenomenon of fluorescence has also been called upon to explain the action of chlorophyll. The wave length in the fluorescence of chlorophyll is increased from that of green to that of red. If the chlorophyll only had the power of shortening the wave length from green to that of ultra-violet, then the ultra-violet thus produced would be the effective light in making the formaldehyde and all would be clear. Unfortunately the organism does not always conform to either our wishes or our hypotheses.

The work of Willstätter, Baly, and others has made all these previous explanations unnecessary. As seen from their equations, chlorophyll is *primarily* neither a screen, a sensitizer, nor a transformer of wave lengths. It takes a prominent part in the series of chemical reactions in the chain of events which results in the production of the formaldehyde. It is a kind of light-energized catalyst, and takes a direct part in the chemical changes during which a large part of the energy represented by the carbohydrate products is absorbed. Its chief rôle is chemical, but this does not preclude the possibility that it may also act as an absorbing screen, which it certainly seems to do.

Part Played by the Carotinoids.—As seen from the equations of Baly, carotin and xanthophyll both have an important rôle in photosynthesis. Chlorophyll B contains more oxygen than A, and xanthophyll contains more than carotin, which facts are related to the series of oxidations and reductions found to take place. Carotin reduces chlorophyll B and becomes xanthophyll. A reducing enzyme then changes xanthophyll back to carotin and the freed oxygen is evolved. This oxygen comes originally from the carbon dioxide that united with chlorophyll A to form B and formaldehyde; while the carotin then changes B back to A by removing the excess oxygen. This continuous process thus results in the intake of carbon dioxide and the evolution of oxygen. The reader must be warned again, however, that these equations are purely theoretical and that we are forced to admit that at the present time we are not at all sure on these points.

The Condensation Process and the First Sugar Formed.—Just how much of the synthesis of carbohydrate material is *photo*synthesis and how much is independent of light has not yet been determined, but the suggestions of Willstätter, Warburg, and others have helped much to clarify our ideas of this matter. As a result of their work, the present tendency is to consider that the *photo*synthesis is over comparatively early in the process. The dark or Blackman reaction and all that follows is post-*photo*synthetic, in that light is no longer necessary, but the exact division point between the photochemical and the dark reactions still remains to be settled.

It is also a disputed point as to what sugar is formed first from the formaldehyde. A few workers like Brown and Morris held that cane sugar was the first to be formed and that hexoses arose later for translocation or respiration purposes; but the majority of workers favor the idea that hexoses are formed first. Not only is this theoretically sound, but Weevers (1924) found that in variegated leaves the green parts contained both hexoses and sucrose, while the yellow portions contained only the higher sugar (sucrose). Likewise when *Pelargonium* was kept in the dark and then exposed to light, chemical analyses showed that the first sugars to appear were hexoses, followed by sucrose, and then starch. Clements (1930) came to the same conclusions after studying the hourly variations of the carbohydrates in leaves and petioles.

An objection raised to the hexose theory is that if one hexose, e. g., dextrose, is formed, then there must also be formed fructose in order for sucrose to be formed later, since (Chap. XII) cane sugar (sucrose) is made from these two hexoses. The sugars, however, are notoriously unstable, and Nef found that if one begins with dextrose and NaOH, no less than 93 substances are ultimately in equilibrium in the solution! Hence the problem of the origin of another hexose is not a very serious one.

Two molecules of formaldehyde may possibly unite to form glycolic aldehyde, a sugarlike substance: $CH_2O+CH_2O=CH_2OH—CHO$. This in turn may combine with formaldehyde to form glyceric aldehyde, a triose, with distinct properties of sugar:

$$CH_2OH—CHO+CH_2O=CH_2OH—CHOH—CHO.$$

Further condensations of trioses to hexoses are not at all difficult in the laboratory. None of these condensations requires any consumption of energy, and the change from formaldehyde to sugar is probably nothing more than the sum of the various reactions already well known *in vitro*.

In **conclusion,** we may feel moderately certain that the secrets of photosynthesis are revealed in their broader phases, although one must ever keep in mind that the simple *possible* schemes worked out in the light of our present physiological knowledge may not be the real ones. Neither are those *in vitro* necessarily the ones employed by the living plant.

QUESTIONS

1. Why is the formaldehyde produced in the process of photosynthesis not poisonous to the plant?
2. What are the present possibilities of producing food chemically?
3. What is catalase? What is meant by *in vitro?*
4. Using the equations of Baly, how many grams of glucose could be produced from a gram of carbon dioxide? How many grams of water would be required?
5. What is meant by condensation? polymerization?

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CHAPTER VII

OTHER WAYS OF PROCURING FOOD AMONG THE HIGHER PLANTS

All the world is little else in nature but parasites and sub-parasites.
—B. JONSON.

Classification of Plants.—The plant kingdom is divided into four major divisions upon the basis of the structure and life history of the plants concerned. These four divisions also differ in the length of time that they have been upon the surface of the earth. The simplest and least differentiated are the oldest, while the more complex and most highly specialized are, in general, among the newcomers on the earth.

The oldest and simplest plants comprise the division of the *Thallophytes,* which are plants without true roots, stems, or leaves. They have developed along two diverging lines, one subdivision comprising the *Algæ,* which includes seaweeds, pond scums, and other simple water plants. The Algæ all contain chlorophyll and are able as a result of this pigment to live independently of other organisms. The *Fungi,* on the other hand, which are included in the remaining subdivision of the Thallophytes, lack chlorophyll and are consequently unable to manufacture their own organic food. They will be discussed more fully in the next chapter.

The second oldest division of the plant kingdom includes the *Bryophytes* or mosses and their allies, the liverworts. They are the simplest land plants and, in practically all cases, are able to manufacture their own food.

The *Pteridophytes* form the third division of the plant kingdom and include the ferns, scouring rushes, quillworts, and club mosses. Here true roots, stems, and leaves are well established. The coal deposits are largely made up of the carbonized remains of members of this group.

The most highly specialized and most recent plants form the great division of the *Spermatophytes,* or seed-bearing plants. Most of the plants of high economic importance are in this group, which are now the dominant land forms and contain species well adapted for the great variety of climatic conditions found at various places on the surface of the earth, ranging from the moist, hot tropics to the cool, subarctic regions and the dry, nearly barren deserts.

Autotrophic Plants.—By far the great majority of the Spermatophytes are *autotrophic*, i. e., they are able to manufacture their own organic food with the aid of the chlorophyll they contain. A few, however, have lost this power and have become dependent either entirely or partially upon food produced by other organisms; such plants are said to be *heterotrophic*. From what has been previously said, it is clear that photosynthetic power depends upon the presence of chlorophyll, and only those seed plants can manufacture their own food which contain this necessary pigment.

Saprophytes.—Now it is obvious that a plant which does not make its own food must get it from one of two sources,—either from dead organisms or from living ones. A few of the Spermatophytes are equipped for the former habit, and live from the organic matter contained in decaying plant and animal matter. The Indian pipe (*Monotropa*) is a familiar example in rich woods, where it is found living on the food to be obtained in humus. From the white stems, which lack chlorophyll, it has received the name of "ghost plant." Since it does not make its own food there is no need for leaves, which accordingly are reduced to mere scales. Such plants which obtain their organic food from *dead* sources are called *saprophytes*.

Symbiosis.—Plants which get their nourishment from *living* organisms are known as *parasites* and illustrate one phase of *symbiosis*, which is the living together of any two organisms of two different species. The word [from *syn* (Gr., together) and *bios* (life)] has no reference to the nature of the relationship and implies nothing whatever as to the benefits to be derived from the union. There are hence two types of symbiosis. In the first instance we shall consider, the union may be of mutual advantage, in which it is impossible to say that one of the symbionts is receiving more from the partnership than the other. This is *mutualism*. True mutualism among the seed plants is very rare, although many cases exist between seed plants and fungi and will be discussed in the following chapter.

Grafting.—In grafting, a branch or bud of one plant is united to the stem of another. This union between the two stems is made in such a way as to bring the greatest possible amount of the meristematic tissues (cambium) of the two plants together, with the result that the two stems grow together and form one plant. The root of the *stock* secures water and mineral salts for the entire plant, while the leaves of the *scion* carry on photosynthesis for both scion and stock. This seems to be as near a case of genuine mutualism, where both plants profit equally, as can be found among the Spermatophytes.

Parasitism.—Generally one plant of the union benefits more than the other. If the parasite contains no chlorophyll and gets all of its food from the *host* (an ironic term) or plant upon which it grows, it is said to be a *complete* parasite. Among the complete parasites of the seed plants are the dodder (*Cuscuta*) which grows abundantly on clover, nettles, buckwheat, and many other herbs. The young seedling obtains enough nourishment from the seed to grow until the plant is able to attach itself to a neighboring host, when the roots die severing the connection with the ground; and from then on both food and water are obtained through the *haustoria* or "suckers" sent into the host. *Orobanche,* which grows on ragweed, *Epifagus* (beechdrops) on beech roots, and *Conopholis* (cancer-root) on the roots of oak are other fairly common examples of complete parasites. *Rafflesia* of Java grows on the roots of trees and sends to the surface only the flower, which, *mirabile dictu,* is the largest flower known,—over three feet in diameter.

The mistletoe, which covers the branches of mesquite, poplars, oaks, and other trees in the southern and southwestern United States as well as in various parts of Europe, bears fleshy leaves with a pale green color indicating the presence of some chlorophyll. To what degree this plant is independent of the host for its food supply is not certainly known, but it cannot be a complete parasite. Kostychev showed that in some cases the mistletoe may carry on photosynthesis as actively as autotrophic plants, indicating that only water and salts are obtained from the host. And Weir (1916) found that the dwarf mistletoe might even manufacture some food for the plants on which it was growing. Six lodgepole pines on which this plant occurred and (at the same time) six healthy noninfested trees were defoliated. Two years later, those with no mistletoe were dead, while the infested trees were still alive! The pale green color of the leaves and their paucity in chlorophyll, however, indicate that the mistletoe is normally at least a *partial* or *semi*parasite. Such plants, which make a part of their food and get the rest from other sources, are also said to be *mixotrophic*. To this same group belong several members of the figwort family, e. g., *Euphrasia*, *Pedicularis*, and *Odontites*.

Gradations.—Between complete and partial parasitism and between parasitism and mutualism are to be found all possible gradations. It is often difficult to say just how much advantage each member of the partnership is reaping from the relationship, so that there are all manner of intermediate stages possible between complete parasitism on one hand and perfect mutualism on the other, with partial parasitism as one of the intermediate steps. *Helotism* (slavery) is such an intermediate condition in which one member of the firm seems to profit while the other continues its existence with neither appreciable gain nor loss. Helotism is more common among the lower plants than in the Spermatophytes, but if the chloroplasts *were* captured one-celled organisms, as has been suggested (Chap. V), this would be a helotic relationship.

Man and his domestic animals furnish analogies in the animal kingdom of these same relationships which illustrate the difficulty of determining exactly where mutualism stops and parasitism begins. Is man's relation to horses mutualistic or parasitic? Does a cow profit more or less than a horse from her human relationships? In fact, man's relation to woman has gone through many of these stages. Among barbaric peoples the wife was a slave who was dependent for her very existence upon the caprices of her husband and master. This was pure helotism. Gradually the position of woman rose among civilized peoples until the twentieth century, when the marriage relationship had reached the phase of mutualism, each partner profiting equally. In these modern days, however, when woman has taken over many of the privileges of man without showing a willingness to assume at the same time his responsibilities, marriage seems commonly to have become a form of parasitism. Continuing this analogy, saprophytism is living from life insurance.

Facultative and Obligate Saprophytism and Parasitism.—Some organisms can live either heterotrophically or independently, that is, they have the power to make their own food but may, if conditions are suitable, also get nourishment parasitically from living organisms or as a saprophyte from dead organic matter. A plant which can live only as a saprophyte is said to be an *obli-*

gate saprophyte; if it can live also by some other means, it is a *facultative* saprophyte. Many of the higher plants can use sugar in solution and, in this sense, they are then facultative saprophytes. In a similar way a plant which *must* live parasitically is an *obligate* parasite; while, if this is only one method of obtaining a living, it is only a *facultative* parasite. Thus the same plant may be a facultative parasite and saprophyte at different times in its life.

Sporophyte and Gametophyte.—In the evolution of the plant kingdom, the appearance and development of the sporophyte generation, accompanied by the corresponding decrease in the gametophyte, are connected with important changes in the methods of securing food. In the Thallophytes, where a sporophyte generation can hardly be said to exist, the gametophyte is autotrophic in the Algæ and parasitic or saprophytic in the Fungi. In the Bryophytes, the well-organized sporophyte is for the most part parasitic upon the gametophyte; it makes little or no food and depends upon the chlorophyll of the gametophyte for its sustenance. In a few of the mosses, the stalk of the sporophyte (seta) contains some chlorophyll, indicating that it is partly autotrophic; but, with the exception of a few Algæ, not until the Pteridophytes are reached, are entirely independent sporophytes produced, the gametophyte (prothallium) at the same time still retaining its independence. The gametophyte of the *higher* Pteridophytes, e. g., *Lycopodium*, has almost completely lost, however, its autotrophic nature. The chlorophyll-bearing tissue of the gametophyte gets less and less as the chlorophyllous tissue of the sporophyte increases. It thus becomes more and more dependent, and finally finishes in the Spermatophytes as a complete parasite.

The young seedling begins life as a saprophyte, using the food stored in the seed by the previous generation. As the plumule unfolds exposing the leaves, an increasingly larger proportion of the food is made by the plant, which thus becomes more and more independent as the stored food decreases. Hence the one individual within the short space of ten days during germination goes through all the stages from obligate, complete saprophytism to complete autotrophism.

Injury Due to Parasites.—Plant diseases are generally associated with physiological disorders. One type of plant disease is produced by an improper physical environment; the plant lacks light, the proper mineral salts, or some other essential to normal growth.

But by far the most troublesome plant diseases are produced by parasites which attack economic plants and thus cause millions of dollars of damage annually. There are four distinct kinds of injury produced by parasites:

1. While not causing any especial trouble of a localized nature, they may weaken the host to such an extent by reducing its food supply, that it cannot resist unfavorable conditions such as drouth, cool weather, etc. The food supply is so nearly exhausted and so small a surplus can be maintained that the first onset of unfavorable conditions results in the death of the host.

2. Some parasites stimulate local growth so that large abnormal outgrowths or excrescences develop. The "witches brooms" so common on hackberry (*Celtis*) trees, peach curl, and black knot of plum are examples of such growths produced as the result of attacks by parasites.

3. Some parasites, such as the one causing fire blight of pear, produce toxic substances which, when present in the tissues, cause the death of the parts affected. Wherever this disease has gained a foothold, the leaf dies and ultimately the tree.

4. Other parasites bring about the death of the host by interfering with some general and necessary function such as the water conduction. By plugging up the vessels of the wood which carry the water from the roots to the leaves, the general water supply from the soil is shut off and the plant dies from lack of water. Although in the past this has been supposed to be the action of the so-called "wilt" diseases, it is now known that the majority of these diseases produce the death of the plant through the production of toxins. They thus belong in group (3); but this does not preclude the possibility that a part of the damage done in some cases may be connected with the water supply.

Mixophytes.—In a previous section mention has been made of the possibility of manufacturing food and at the same time using food already elaborated. There is no good reason why any green plant should not be able to use organic food if supplied to it in solution or if it possesses enzymes capable of making the food available; and some plants have special structures to enable them to supplement their normal supply of organic food, especially nitrogenous compounds. Such are the insectivorous plants, which include the pitcher plants (*Sarracenia* and *Nepenthes*), the sundew (*Drosera*), Venus's flytrap (*Dionæa*), and the bladderwort (*Utricularia*). These plants, which are either bog plants, water plants, or epiphytes, contain chlorophyll but at the same time are provided with a means for catching or holding small animals, mostly insects, which are later digested.

In the pitcher plants (Fig. 4A) all or a part of the blade is modified into an urn or pitcher which fills with rain water, in which any venturesome insect is drowned and subsequently digested. Hairs pointing inward and downward prevent the escape of the prey.

The sundew (Fig. 4B), characteristic of peat bogs, has a leaf blade covered with pin-shaped glandular hairs that secrete at their tips a digestive enzyme. When an insect alights upon the leaf, it is held fast as the hairs bend in toward it. At the same time, the digestive enzyme continues to be secreted until all utilizable portions have been absorbed, when the hairs open up to their original position. The sundew is also able to digest lean meat and egg white placed upon the leaf.

In *Dionæa* (Fig. 4C) the end of the blade is divided by the midrib into two hinged valves bearing long teeth along each margin and three sensitive elastic bristles on the upper surface of each half. When an insect alights upon the leaf and touches a bristle, the two halves fold together very quickly thus imprisoning the insect, which is later digested by an enzyme secreted from glandular hairs on the leaf surface.

Utricularia (Fig. 4D) is a submerged rootless plant which bears on the branches as modified leaves numerous hollow bladders, which have only one entrance and that closed by a kind of trapdoor opening only inward. Small water animals which are drawn into the bladder through this entry are thus shut off from escape and are later presumably digested. Although Stutzer (1926) thinks the digestion is brought about by bacteria in the bladders, which probably take some of the food for themselves and thus furnish another example of symbiosis, Adowa (1924) found proteases present, which he thinks came from the *Utricularia* itself.

Although the insectivorous plants are among the few which are especially adapted for supplementing their food supply in this fashion, there are other autotrophic plants which can use organic food. It has even been suggested (Christy, 1923) that the common teasel (*Dipsacus*) augments its food supply in a manner

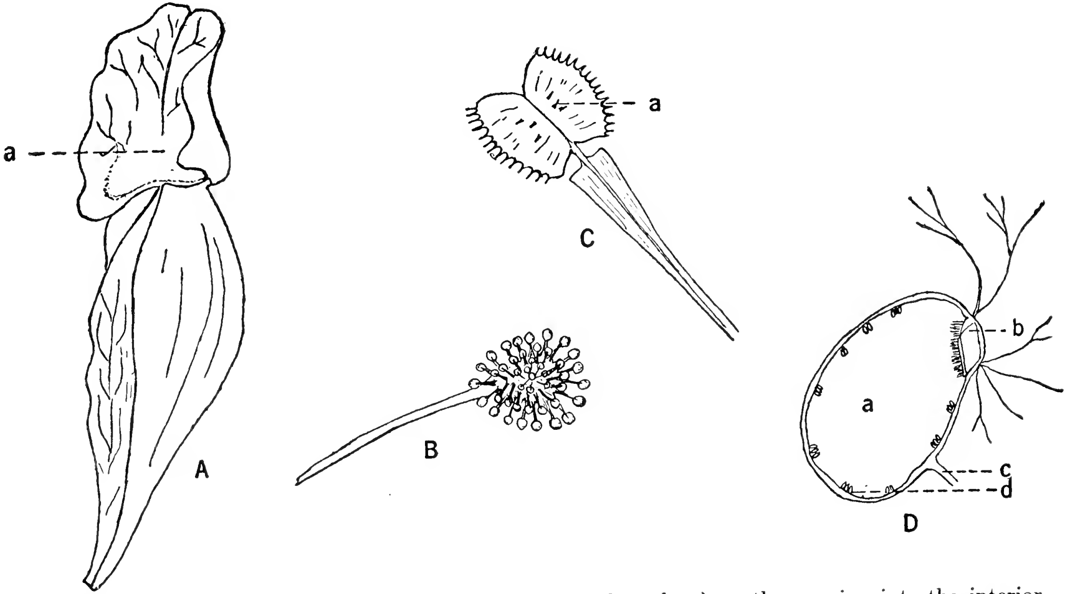

FIG. 4.—Insectivorous plants. A, *Sarracenia* (the pitcher plant); a, the opening into the interior. B, *Drosera* (the sundew). C, *Dionæa* (Venus's flytrap); a, indicates the sensitive hairs. D, *Utricularia* (bladderwort); a, is the interior of the leaf; b, the opening and valve; c, the stalk; and d, groups of secretory cells which aid in digestion.

similar to the pitcher plant by digesting insects drowned in the water that collects in the bases of the connate leaves. Also Zambelli (1929) says that *Petunia*, with the aid of the glandular hairs on the aërial parts of the plant, captures insects and digests them by means of proteolytic enzymes secreted by these same hairs. It is a common observation that green algæ such as *Ulva* are especially common around the outlets of sewers where the water is rich in organic matter. Many algæ have been cultivated in the dark when organic food was added to the nutrient solution, thus changing over directly from autotrophic plants to saprophytes. Saposchnikov (1891) showed that *Dombeya* leaves which were freed from starch in the dark formed about 5 grams of starch per square meter of leaf surface in seven days when floated upon a 20% solution of cane sugar; and peanut embryos can develop for some time on various sugar solutions.

Roots are able to absorb sugar much more easily than leaves, and Knudson (1916) and others have shown that corn, beans, radishes, etc., can supplement their carbohydrates by absorbing sugar from sugar solutions. Although absorption is favored and augmented by light, it will take place in the dark, and it is quite possible to raise seed plants in darkness for the entire period of their lives on solutions of sugars and other nutrients.

Breazeale (1923) also has shown that roots can absorb the carbonate ion from the soil carbonates, but he thinks there is no reason to suppose that the carbon thus absorbed replaces to any extent that absorbed from the air. The work of Stoklasa (1927) and others cited in Chapter III may require us, however, to readjust our conclusions on this matter.

Relation of Photosynthesis to Plant Structure.—In concluding this subject of photosynthesis, it is fitting at this place to remind the reader that, while it is impossible to say that structure determines function more than function determines structure, nowhere else is the interdependence of these two more clearly seen than in this matter of photosynthesis. The important points in their structure which separate plants from animals, as has been pointed out by Ganong, are all connected with their food supply and the ability of the green plant to elaborate not only its own food but that for all other organisms as well:

1. Animals must get their own food from outside. Plants can manufacture it from materials which circulate by them. These

food materials,—carbon dioxide and water—while not always abundant are very widely distributed and in constant motion. Thus animals are, in general, capable of moving from place to place while plants are, for the most part, stationary.

2. These materials which are used in the construction of food by the green plant come in towards it from all directions, which accounts for the radial symmetry of the plant, with its branches and roots passing out in every direction.

3. The carbon dioxide and water, while present in sufficient quantities, are not abundant. For this reason it is advantageous to expose as much surface as possible, hence the profuse branching of both stems and roots.

4. Light is quickly absorbed and penetrates only a short distance. This necessitates structures with a broad surface, which are at the same time thin. Leaves fulfill these requirements admirably and enable much material to be spread out into a thin absorbing surface.

5. The light and carbon dioxide come from above, while water and mineral salts come from below. This results in the formation of a system for absorbing mineral salts and water from the ground, while an aërial system is also required for receiving light and carbon dioxide. Thus arises the division into root and shoot.

6. It is advantageous for the leaves to get up into the light as completely as possible. At the same time connection must be maintained with the water supply below, which explains the origin of the various types of stems.

This is sufficient to show the close connection between the photosynthetic function and the structures of plants as we now find them. There is always the tendency to read into such beautiful harmonies purposeful adaptations, but one must ever remember that the successful well-adapted structures that cover the earth at the present time are only a small fraction of the experiments of nature. Only living people ever praise the physician.

QUESTIONS

1. Some plants have variegated leaves with white margins. How do such white parts get their food?
2. A noted horticultural firm has offered $1,000 to any gardener who will produce a white-leaved geranium. What are the chances for success?
3. What are the requisites for a successful graft?

4. Are parasites and saprophytes ever beneficial to man?

5. Trace the development of the gametophyte from the Thallophytes to the Spermatophytes.

6. What is the *biogenetic* law? Illustrate.

7. Which is more important, structure or function? Why?

8. Does the scion ever have any effect upon the stock or the stock upon the scion in a graft? (See Chap. XXX and the article by Hofman.)

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CHAPTER VIII

NUTRITION IN THE LOWER PLANTS; CHEMOSYNTHESIS

> Death rides in every passing breeze,
> He lurks in every flower,
> Each season has its own disease,
> Its peril every hour.
> —R. Heber.

The Fungi.—Although this book is primarily concerned with the physiological problems of the higher plants, it has been deemed excusable and profitable, either because of their great economic importance or because of their interest from the viewpoint of general physiology, to introduce here a chapter on the special physiology of some of the Thallophytes, notably the fungi, which contain no chlorophyll, and in the majority of cases must derive their energy from organic compounds as described in the previous chapter. They live either parasitically or saprophytically, the same classification applying here as in the dependent seed plants. Among the saprophytes are to be found three groups of outstanding importance, —the yeasts, the molds, and the bacteria.

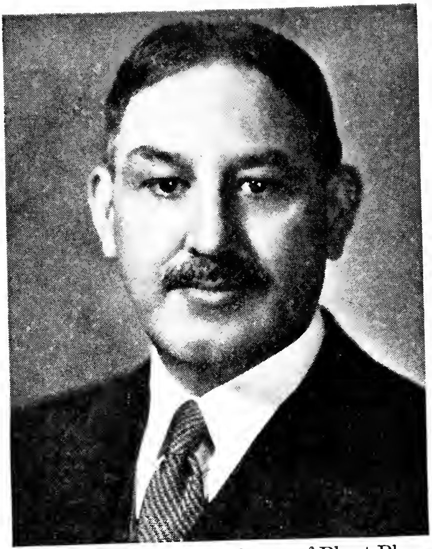
The Saprophytes.—The yeasts are simple one-celled plants, which grow especially well on sugars. They are found almost universally in nature and are cultivated for their use in two important industries—brewing and baking. The sugars found in beer wort, grape juice, and other fruit juices supply them with the food necessary for their growth and rapid reproduction. They will be discussed further in Chapter XXV.

The molds are another group of saprophytic organisms. These filamentous plants so common on fruits, vegetables, etc., are not very exacting in their nutrition, but are able to use a wide variety of organic food. Certain saprophytes are much more limited in their nutritional requirements than others; some can grow and thrive on only one kind of food while others can be grown practically anywhere. The members of the former group thus lend themselves to the method of "selective culture" in isolation experiments.

The saprophytic bacteria make up what are called the "bacteria of decay" and, although they may cause vile odors and tastes, they are, on the whole, beneficial to man. They cause the return of organic matter to the inorganic state, where it can again be used. If it were not for these agents of decay, when an organism died the complex molecules of organic matter would remain in a condition where they would be unavailable for the use of other organisms. The dead plants and animals would long ago have smothered out life on the surface of the earth, and the world would in a peculiar and fitting sense be "dead." The saprophytic bacteria and allied forms, however, are able to continue the organic cycle and to derive their energy in the process.

The Symbionts.—The symbionts may similarly be divided into two groups: those which live mutualistically and those which are parasitic. Among the important symbionts are the *mycorrhiza* which consist of a web of fungal filaments that surround the roots of a plant and aid it in obtaining mineral nutrients from the soil. These have been found to be of two types. In the *endotrophic* mycorrhiza as found in many of the halophytes (*Plantago coronopus*, *Glyceria maritima*), in many heaths (*Andromeda*), in black maple (*Acer nigrum*), horse-chestnut (*Æsculus*), and walnut (*Juglans*), the fungal filaments penetrate the cortex of the root and enter the cells curling up inside. In the *ectotrophic* mycorrhiza of the birch (*Betula*), hickory (*Carya*), beech (*Fagus*), oak (*Quercus*), and sugar maple (*A. saccharum*) the hyphæ cover only the surface of the roots and do not penetrate into the interior. Mycorrhiza are of very common occurrence, and forest trees growing in rich humus seem generally to be associated with such fungi.

The fungus obtains organic food from the tree and in turn gives it water and mineral salts. The soil, under the conditions found on the forest floor, is a seething mass of bacteria, higher fungi, and other microörganisms. This living environment of the roots makes the competition for mineral salts especially keen, since all the organisms present in the soil need a certain amount of the available salts. It thus appears that plants with mycorrhiza associated with them have a much better chance to compete with the soil organisms than those which contain no mycorrhiza. In this way both the tree and the fungus seem to be benefited, although some investigators think that the relation is much to the



Dr. C. A. Shull, Professor of Plant Physiology, University of Chicago. *Respiration. Dr. Shull, through his connections with "Botanical Gazette" and "Plant Physiology," has been very influential in the "physiological press" of the United States.*

Dr. R. Willstätter, Professor of Biochemistry, University of Munich, Germany. *Chemistry of chlorophyll and related pigments; Enzymes.*

advantage of the fungus and that mycorrhiza are more parasitic than mutualistic.

In some cases, e. g., the heaths, the green plants are possibly dependent upon the presence of the fungi. This is said by many workers to be the case with *Calluna.* Here the entire plant may be permeated by the fungal filaments and when the seeds are formed some of the mycelial strands are already present, ready to develop parallel with the plant. This obligate condition is considered of value to *Calluna* in that the fungus can fix free nitrogen (Chap. IX) and also aids the heath to absorb soluble compounds from the acid soil where it grows. Knudson (1929), however, insists that this plant can grow without the mycorrhiza if the acidity and iron nutrition are handled properly. While this may be true in cultural conditions, this does not preclude the possibility that the two are valuable to each other as found in nature. A similar situation may be true in regard to the relation between some orchids and their mycorrhiza; they *can* get along without the mycorrhiza if the proper conditions are met, but the mycorrhiza, nevertheless, aid them in nature where the "proper conditions" happen to be lacking. In the case of *Cattleya,* the fungus seems to assist in the mobilization of the reserve foods in the seed at the time of germination.

The dependence of plants upon their mycorrhiza may explain in part why such plants are extremely hard to transplant. When taken from their native environment, the young roots are likely to be broken off and with them the mycorrhiza. They are then often placed in a soil which may lack completely all of the cooperating fungi, in which case death is almost sure to result. Such transplantings should be made with due care to keeping intact as many of the feeding roots as possible, and some of the same soil from the original locality may profitably be placed around the roots in the new location. It might be added at this point that Martin-Zédé (1922) showed that success in transplanting was connected also with the orientation of the tree. When trees were oriented as they had been before, the mortality was only 7%, while if they were placed in a different position in regard to the points of the compass, 50% died.

The bacteria which live in the nodules on the roots of legumes (Chap. IX) are another excellent example of mutualism. These bacteria receive organic food from the legume and in turn produce nitrates, which the plant can utilize either before or after the death of the nodule-bearing rootlet. Lineberger (1926) reports a symbiosis of somewhat similar nature between the hydrophyte, *Azolla*, and the alga, *Anabœna*. The latter lives in the leaf cavities of the former and there seems to fix nitrogen, which the *Azolla* can later use. If there is already plenty of nitrates in the solution, the *Anabœna* may become a bit of a nuisance but never an actual pest.

Lichens are plant complexes consisting of a fungus and an alga living in such close conjunction that the resulting structure behaves as an organic unit. They are found on trees, boards, rocks, and similar places where the supply of food materials would seem to be rather meager. Formerly they were considered to be individual plants but, when it was discovered that the alga and fungus could be grown independently, the dual nature of the organism was recognized. When grown separately the fungus differs more than the alga from the condition found in the dual relationship of the lichen, thus indicating that the fungus is more dependent upon the alga than the alga upon the fungus. The exact nature of the relationship is somewhat in doubt. It is commonly thought to be one of helotism, the alga profiting and losing about equally by the association, while the fungus apparently obtains its organic food supply from the alga and thus profits considerably. Others hold to the opinion that it is genuine mutualism in which the alga is protected and enabled to live in drier habitats than it otherwise could. At the other extreme is the opinion that the relation is one of pure parasitism in which the fungus robs the alga and reaps all the benefit, giving nothing in return.

Parasitic Fungi.—The fungi which are parasitic include the rusts, smuts, mildews, and some of the bacteria. Here are to be found the forms which cause the large majority of plant and animal diseases. It is worthy of note that man in his conquest over nature is to-day fighting his most serious enemies in the world of microörganisms. He has conquered many of the inanimate forces of nature and bent them to his will. He has defeated the other animals (except the insects), after having invented weapons which made their subjection comparatively easy, but the microorganic population still contains some of his worst enemies, and here some of the most important battles are now being waged.

The measures of microörganic control are two: first, the *cure* of disease, and second, its *prevention.* The preventive measures may all be summed up under the term *prophylaxis* and are similar in their methods whether one is a physician, a veterinarian, or a plant pathologist. Seven methods of preventing disease are in common practice and may be briefly described:

1. *Personal hygiene.* By this is meant cleanliness and the ordinary methods employed to keep the organisms of disease from multiplying or finding refuge on the person. With plants the common method of washing is spraying, in which the plants are covered with a solution of chemicals which prohibits the growth of microörganisms and at the same time does not hinder the development of the plant sprayed. Various sprays are applied differing with the disease to be warded off, the species of plant, as well as with the condition of the plant at the spraying season. A plant in full blossom will require different treatment from one bearing leaves or one with only the bare stems exposed.

2. *Public hygiene or sanitation.* In the combat against human diseases, the maintenance of a pure water supply, of proper drainage, of pure air, etc., are included under sanitation. Similarly with plants, pure air must be maintained and not air full of smelter smoke with its contained toxins. Pure air for a plant is air with a plentiful supply of carbon dioxide but free from carbon monoxide, sulphur fumes, and other poisonous gases. It is the business of the sanitation or public health official to see that infected areas are disinfected as soon as the disease has been checked. Similarly the plant pathologist attends to the burning of diseased plants and takes all measures possible to stop the further spread of the disease. The maintenance of a good water supply, of proper drainage, etc., are all included in the work of the plant sanitary engineer.

3. *Quarantine.* Just as a man who is infected with a dangerous communicable disease is isolated by measures of quarantine, so are quarantine stations provided by the U. S. Department of Agriculture and by the State governments to prohibit the entrance and spread of plants with communicable diseases. The "influenza," which caused more deaths throughout the world in a given length of time than any disease in recent times, was imported from Europe in the summer of 1918. In like manner many of our worst plant diseases have been imported from foreign lands because of an ineffective quarantine. The rice smut came to South Carolina from Japan in 1898. The chrysanthemum rust came from Japan to

America through England in 1896, and the potato blight was imported from Chile to Colorado. All of these destructive diseases could have been prevented by the proper quarantine measures.

4. *Breeding resistant varieties.* This type of preventive measure is obviously more easy to effect in plants and the lower animals than in man. The "human" species, in fact, by its sentimental methods of "humane" treatment is the only one which keeps in the race all the weak and inefficient members of the species instead of permitting the laws of natural selection to take their course. In the plant world, this line of attack, i. e., breeding resistant varieties, has met with very promising results. Hardly a month passes without the announcement of the discovery and development of a new variety especially resistant to some particular disease. Among those developed to date are the anthracnose and mosaic-resistant beans, the black-rot (*Fusarium*) resistant cabbage, wilt-resistant cotton, and rust-resistant wheat.

5. *Vaccination.* In this treatment the individual is given a mild form of the disease which protects against the more virulent form by placing at the disposal of the organism the means of combat which the mild form of the disease has developed in it. Thus there is vaccination for typhoid, smallpox, cholera, and many other diseases. Since plants have no circulatory system which permits of general treatment in this way, vaccines have been used on them to only a slight extent and then with no marked success.

6. *Serum therapy.* In the case of serum therapy an extract or serum is given which counteracts the poison developed by the disease. Serums have been developed for the toxins of diphtheria, tetanus, and hog cholera, but for the same reason given above, this method has not been applicable to plants. Although the serum treatment is commonly used as a cure it may also be given as a preventive, and can hence be listed here among the prophylactic measures.

7. *Antiseptic surgery.* In this category are included all the measures of *antisepsis* which bring about *asepsis.* The sterilization of the instruments and of all bandages, the employment of lotions which prohibit the growth of microörganisms, and all other such operations are here included. In the plant world such prophylactic measures are of importance chiefly in tree surgery. After pruning and cutting, the wound should be well washed out and then covered

with some antiseptic substance such as tar which hinders the entrance and growth of fungi.

Chemosynthesis.—All the forms of plants thus far mentioned, including autotrophic forms, saprophytes, and parasites, have obtained their food from organic compounds built up by means of energy derived from sunlight through the agency of chlorophyll. For all *practical* purposes this is the only way in which food can be manufactured. There is, however, a small group of fungi which, although lacking chlorophyll or an analogous pigment, are nevertheless able to build up their organic food in an entirely different manner quite independently of sunlight. These plants use the energy derived from the *oxidation of inorganic compounds* to build up organic foods, thus exhibiting a physiology of a very different type, which up to the present time has not been thoroughly investigated. This construction of food by means of energy derived from the oxidation of inorganic compounds or by other similar chemical reactions has been called *chemosynthesis.*

The most important of these forms are the nitrifying bacteria (*Nitrosomonas*) of the soil which are able to oxidize ammonia into nitric acid and construct the foods for their own bodies with the energy derived.

$$NH_3+2O_2=HNO_3+H_2O+\text{energy}.$$

Ammonia gives 5.3 calories of heat per gram while starch gives only 4; so that it would not be surprising if the plant not only used the oxidation energy produced (in place of light) for building a part of the necessary food, but also used the ammonia as *food.* This is a phase of the activity of these bacteria which needs thorough investigation. To how great a degree is the ammonia used as *food* and to what extent is the energy derived used as a substitute for *sunlight?* Is this oxidation a substitute for photosynthesis or is the ammonia a substitute for organic food? Winogradski found that when grown in a nutrient medium containing no organic food of any kind the bacteria grew and formed an appreciable amount of organic substance, indicating that we are dealing with a genuine chemosynthesis. Godlewsky (1896) further showed that these same bacteria obtained their carbon from carbon dioxide of the air and not from carbonates in the nutrient medium. As will be emphasized later (Chap. IX) these bacteria play an important part in the nitrogen cycle.

The sulphur bacteria (*Beggiatoa* and *Thiothrix*) which are found in bogs, sulphur springs, and in the ocean, play a similar part in the sulphur cycle and help to keep the sulphur in an available shape to be used by higher organisms. These bacteria oxidize hydrogen sulphide to sulphur and water:

$$2H_2S+O_2=2H_2O+S_2+122.2 \text{ cal.}$$

Here there seems little doubt that the bacteria, which seem so morphologically simple and are yet so physiologically complex, are able to carry on chemosynthesis and then to use the sulphur which results and is stored up inside the cell as a source of energy, oxidizing it later in the presence of water to sulphuric acid:

$$S_2+2H_2O+3O_2=2H_2SO_4+284.4 \text{ cal.}$$

This oxidation takes place only in the presence of sufficient calcium salts to neutralize the sulphuric acid produced, thus forming calcium sulphate: $CaCO_3+H_2SO_4=H_2CO_3+CaSO_4$. The carbonate or carbonic acid released during this neutralization process is then used up by the bacteria to form carbon compounds. These forms are especially important in the ocean where the quantities of organic matter which fall to the ocean floor are gradually decomposed, with the elimination of large quantities of hydrogen sulphide, which, if it reached the upper layers of water, would poison them and make them unfit for life. This the sulphur bacteria prevent. Also in the soil are sulphur bacteria which can oxidize the sulphur of proteins to elementary sulphur, which is either eliminated as such or oxidized to sulphuric acid. *Thiobacillus thio-oxidans* is one such form which has been isolated in pure cultures and which can oxidize sulphur to sulphuric acid. It grows best in a very acid solution (pH 2.5) and will even grow in Normal sulphuric acid. With the energy derived from this oxidation it can take the carbon from carbon dioxide, the nitrogen from ammonia, and thus make its own foods. It may, therefore, represent one of the earliest forms of life on the earth.

Although hydrogen is produced in large amounts, it is found in the air in only small quantities. One explanation of this has been given by Kaserer (1906) and Niklewski (1910), who have showed that there are certain bacteria, which can oxidize hydrogen. From a chemical viewpoint, hydrogen is the best possible source of energy because of its high heat of combustion (34.6 kg. cal. per gram), which makes it more than eight times as efficient in heating as starch. These hydrogen bacteria (*Hydrogenomonas*) are found in the soil and can oxidize hydrogen by means of carbon dioxide in the presence of water into formaldehyde and water:

$$CO_2+2H_2=CH_2O+H_2O.$$

The formaldehyde is then presumably condensed into carbohydrates as in the higher plants. If these bacteria are grown upon a medium containing organic food, they cease to assimilate the hydrogen and become ordinary saprophytes.

Leptothrix and *Crenothrix*, which are found in conjunction with iron deposits (sometimes as a result but more often as a cause), can oxidize ferrous compounds, especially ferrous bicarbonate, into ferric hydroxide and carbon dioxide:

$$2FeHCO_3+2H_2O+O_2=2Fe(OH)_3+2CO_2.$$

The ferric compound is generally deposited in the mucilaginous sheath which surrounds the bacteria, and it is this deposition which in large numbers has caused 90% of the world's bog iron deposits, as estimated by Harder (1919). The carbon dioxide produced by the bacteria is used in building up carbon compounds. Other forms can use ferrous sulphate, which in the presence of lime is converted into ferric oxide and gypsum. The iron oxide remains within the cell of the bacterium and is deposited upon its death, as mentioned above. Thus the Cuyuna and Mesaba Ranges of Minnesota contain an estimate of 4,200,000,000,000 tons of iron ore, all (or nearly all) of which was laid down by iron bacteria. Molisch and Ellis have stated that the iron bacteria can thrive without iron and attribute the deposits to physicochemical processes rather than to vital ones, but the majority of workers in this field agree with Winogradsky that we have to deal here with a group of autotrophic bacteria.

According to Söhngen, there are bacteria which can oxidize methane to carbon dioxide and water, using the resultant energy in a similar manner:

$$CH_4+2O_2=CO_2+2H_2O.$$

Others have been reported which can oxidize carbon monoxide, charcoal, zinc, manganese, etc.; and it may be expected that still others will be found capable of oxidizing other substances commonly found in waters and soils.

The Purple Bacteria.—This group of exceptional sulphur bacteria, in addition to a green pigment resembling chlorophyll (bacteriochlorin), contains also a reddish-purple pigment called bacteriopurpurin. A third pigment (bacterioerythrin) has been described by some workers, but may have been confused with bacteriopurpurin. At any rate, by the aid of these pigments, according to Engelmann, these bacteria can manufacture their own organic food in much the same manner as the green plants. If this were their regular method of obtaining food and energy, this group should be removed from the fungi and placed among the algæ. But these forms seem also to be able to derive their energy from H_2S in the same autotrophic, chemosynthetic manner as the other sulphur bacteria. In fact, they seem to be able to use the oxygen liberated in photosynthesis to oxidize H_2S. This permits them to tolerate very high concentrations of this gas and to thrive where other organisms would perish. These forms seem to be facultative photosynthesizers or chemosynthesizers as the case may be. They, therefore, occupy a very special position and possibly represent (Lubimenko, 1926) transitional evolutionary forms between the chemosynthetic bacteria which lack pigments and the blue-green algæ with pigments, which, although autotrophic, are easily able to use organic food and are thus facultative saprophytes. The facts that bacteriochlorin seems to be an evolutionary step in the development of chlorophyll and that bacteriopurpurin is related to the phycocyanin of the blue-green algæ support this hypothesis.

Summary.—In the previous pages it has been shown that there are several methods of procuring food which may be classified as follows:

I. Energy from photosynthesis.
    a. autotrophic.
        1. from chlorophyll.
        2. from bacteriopurpurin and bacteriochlorin.
    b. heterotrophic—parasites and saprophytes.
II. Energy from chemosynthesis.

The chemosynthetic forms have been shown to be of extreme importance in some cases in the organic cycles. They have also been shown in others, e. g., the iron bacteria, to be extremely important in the production of insoluble iron deposits, thus removing from the waters of the earth materials found in excess. As a source of food supply to man and the higher animals they are of no importance whatever, but the light they throw upon possible developments of life in places devoid of light or before the existence of green plants upon the surface of the earth is extremely important. These chemosynthetic forms are the only organisms independent of light and green plants, which thus permits them to occupy a unique place in nature.

QUESTIONS

1. What is the method of selective culture?
2. Distinguish between antisepsis and asepsis.
3. What is the difficult problem in connection with chemosynthetic bacteria?
4. Which cause more animal diseases—bacteria or higher fungi? Which more plant diseases? Why this difference?
5. What is the relation between the size of bacteria and their high physiological activity?
6. Lichens are greener in damp than in dry weather. Why?
7. Why are lichens thinner than most fleshy fungi?
8. As stated above, the blue-green algæ are among the simplest autotrophic photosynthetic forms. They are commonly found in hot springs. How can this be explained?
9. While it was previously thought that a given lichen could be made from a fungus and any one of several algæ, Chodat (1930) has shown that this is not the case; the lichen association is very specific. Does this throw any light on the nature of the symbiosis?
10. What is meant by the statement (p. 84) that the chemosynthetic forms may "be of extreme importance . . . in the organic cycles?"

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CHAPTER IX

NITROGEN ASSIMILATION; THE NITROGEN CYCLE

> In all things there is a kind of law of cycles.
> —TACITUS.

Protoplasm, which makes up the living material of the plant, contains in addition to carbon, hydrogen, and oxygen, a large percentage of nitrogen. Wheat straw which seems almost pure cellulose has 0.5–1.0% of the dry weight in nitrogen, while as much as 25% of the dry weight of bean seeds is composed of this element. The nitrogen is absolutely essential for the formation of proteins and consequently of protoplasm. In its absence the leaves of plants are generally stunted, and the foliage, which at first becomes reddish, later develops a yellowish, sickly look. An abundance of available mineral nitrogenous compounds seems to favor vegetative growth and to retard the formation of fruiting parts, so that with plenty of nitrogen, there is a rank production of rich green foliage without flowers; but this relation of nitrogen to reproduction will be further considered in Chapter XXXI. Since nitrogen is present in protoplasm, it is to be expected that a deficiency would derange the general metabolism. Molliard (1922) found that respiratory processes were consequently affected and acids were caused to accumulate when plants were grown with little or no nitrogen. Radish plants thus grown contained five times as much acid in the cell sap as those with nitrogen, and sorrel (a very acid plant) was twice as acid.

This general action of nitrogen may be summed up as follows:

1. Forms a component of all protoplasm and, therefore, is necessary for normal metabolism.
2. Increases foliage and, therefore, is especially important for leaf crops (spinach, forage plants, cabbage, etc.).
3. Retards flowering and maturation of plants.
4. Aids in the production of a healthy, green color.
5. Produces succulence and crispness of foliage, thus improving the *quality* of leaf crops like celery and lettuce.
6. Lowers resistance to diseases, insects, and cold weather.

The composition of the air is about four-fifths or 80% nitrogen. In addition there is also present in the air considerable quantities of ammonia,—about 2.4 mg. in every 100 cubic meters of air. But in spite of the abundance of nitrogen and nitrogenous compounds in the air above the green plants, they are quite incapable of drawing from this vast storehouse. The only important sources of nitrogen for the green plant are the nitrogenous compounds in the form of soluble salts in the soil; and such nitrogenous compounds must be added to the soil supply of soluble materials in some manner if the plant is to profit. This was first demonstrated by Boussingault (1860), who grew various kinds of plants in a sterile soil free from nitrogen. The culture pot was placed in a shallow dish supported above the bottom of a larger vessel, in which the bell jar covering the plant was placed. This larger vessel contained sulphuric acid to prevent the entrance of ammonia, and two tubes which led into the jar from the outside pro-

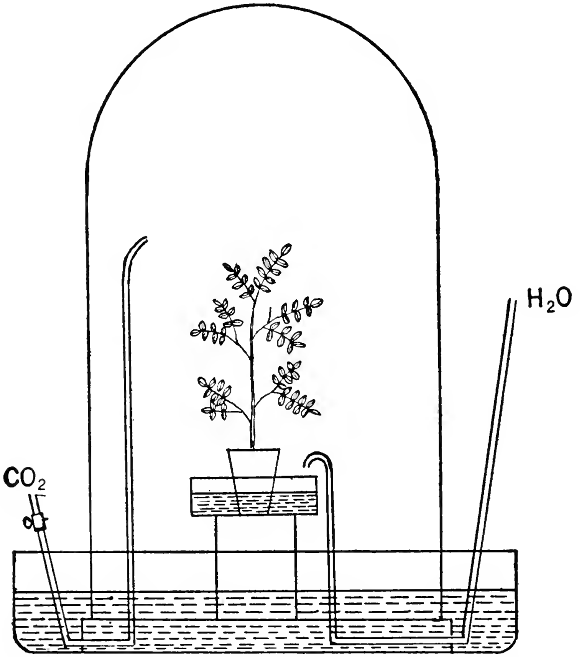


Fig. 5.—Boussingault's experiment. Growing a plant in soil free from nitrogen and air free from ammonia. The pan at the bottom contains sulfuric acid to form a seal.

vided the necessary carbon dioxide and water (Fig. 5). There was no source of nitrogen other than that in the air surrounding the plant, and the amount of initial nitrogen in the seed was determined by the analysis of similar seeds. The apparatus was then exposed to the light and the plant allowed to grow for two or three months, when the nitrogen content was again determined; but under these conditions no increase in the amount of nitrogen was ever detected.

Soil Nitrogen.—The nitrogen in the soil occurs in three different forms. It is found in *organic* compounds as the result of the large masses of organic matter which are constantly being returned to the soil after the death of the organisms of which it was a part. These humus compounds are of various degrees of complexity. The second source of nitrogen in the soil is formed by the *ammonium* salts, which result either from the decomposition of organic matter in the soil, from animal excreta, or to a small degree from the natural processes which operate in the air, such as the evaporation of water, during which a small amount of nitrogen combines with hydrogen forming ammonium compounds. Thirdly, there is the *nitric* nitrogen or that which is in the form of nitrates and nitrites. In these inorganic compounds of nitric or nitrous acid, the nitrogen occurs in the acid part of the salt, where it is united to some base such as sodium or potassium forming sodium and potassium nitrate. This nitric nitrogen is constantly increasing as the other forms decrease.

The organic compounds are not available for the higher plants, which are quite unable to use complex nitrogen compounds such as proteins until they have been broken down into amino acids and nitrates. Whether this is due to the inability of the root to absorb large molecules or to some other cause is not known.

The ammonium salts are of doubtful value in the direct nutrition of plants. In some cases they can be used but not so easily as nitrates. In other cases they seem to be even more available. Up to a certain concentration ammonium sulphate is said to be better than potassium nitrate for peas and other legumes. Corn is said to do best when young on ammonium compounds, and Nagaoka (1904) reported that rice did as well on ammonium salts as on nitrates. The availability of ammonium is probably dependent upon (1) the nature of the soil, (2) the concentration of the ammonium salt, and (3) the nature of the salt. If the soil contains plenty of lime, ammonium salts can be used more advantageously than if the soil is acid, which may be explained by assuming that the ammonia, before absorption by plants, is first oxidized to nitric compounds and forms too much acid in the soil in case no lime is present to neutralize it. Another explanation, however, is that the ammonium salts are absorbed as such, but the basic part of the salt is absorbed more than the acid part, leaving an excess of acid in the soil, which the lime, if present, neutralizes. In low concentrations ammonium salts seem to be as easily used as nitrates, but in high concentrations ammonium salts are toxic. As to the third point, i. e., the nature of the salt, ammonium sulphate is less available than ammonium nitrate. In the case of the former, the ammonium ion is taken up more rapidly than the sulphate ion, with the result that the soil solution becomes physiologically acid. The nitrate ion, however, is absorbed at about the same rate, so that when ammonium nitrate is used, the soil solution does not change appreciably towards either the alkaline or acid side. There is no doubt but that plants may thrive on ammonium compounds, but it is still doubtful whether these salts in nature are absorbed as ammonia or only after transformation to nitrates. Probably most of the compounds are changed into nitrates before being absorbed, although Müntz and Mazé (1898) as well as later workers showed that plants are able to grow and increase in nitrogen content when nourished with ammonium salts on *sterile* soil where no transformation into nitrates can occur. And Prianishnikov (1923), with a revolutionary spirit characteristic of his country, has asserted that nitrates before being absorbed are always reduced to ammonia!

Nitrates, which in general are the best source of nitrogen for plants, are constantly being formed at the expense of the other nitrogen compounds present. Boussingault sealed up a carboy of soil in 1859 and reopened it 12 years later. He found that both the nitric acid and nitrates had increased to more than 200 times the original amount, while the total nitrogen remained approximately the same, showing that this increase of nitric nitrogen had been at the expense of the ammonia and the organic compounds.

Although nitrates are constantly being formed, there is always only a small amount present in the soil. To be sure, some is being removed by growing plants, but this does not account for all the nitrate loss. If a solution of ammonia and nitrates is filtered through a soil, the reason for the loss of nitrates is apparent. The ammonium compounds are found to be absorbed by the soil particles and do not leach through, while the nitrates are held only slightly by the soil and are easily carried away by the water. The organic nitrogen compounds and the ammonium salts thus behave as a kind of reservoir of nitrogen. They are broken down or transformed into nitrates slowly and thus furnish a gradual supply of available nitrogen for the plants growing in the soil.

Nitrification.—How does this transformation from ammonia to nitrates occur? Schlösing and Müntz (1877) found that, if water containing ammonia was allowed to percolate slowly through sand, the filtrate contained more nitrate than the original! If chloroform vapor was allowed to permeate the sand, however, the nitrification was slowed up and ultimately stopped. Now chloroform probably had no chemical action, and, since it was known to have a direct effect upon living organisms, it was concluded that nitrification was a process depending upon microorganisms. This conclusion has been verified many times since. Winogradski (1890) has been given the credit for the original isolation in pure culture of the nitrifying organisms, although there has been some doubt thrown on the purity of his cultures and the reliability of his culture methods.

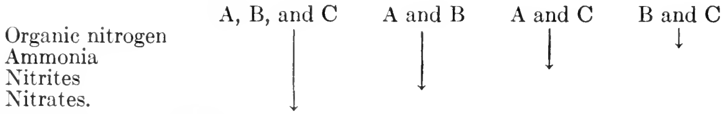
Winogradski further showed that nitrification is not a simple process in which only one genus of bacteria is involved but that there are two distinct steps in the process. One group of bacteria (*Nitrosomonas* and *Nitrosococcus*) oxidizes the ammonia as far as *nitrites* only, while a second genus (*Nitrobacter*) continues the process and oxidizes the nitrites to nitrates.

Nitrosomonas and Nitrosococcus: $2NH_3 + 3O_2 = 2HNO_2 + 2H_2O$
Nitrobacter: $2HNO_2 + O_2 = 2HNO_3$.

Nitrosomonas is a small, oval, motile form, characteristic of the soils of Europe and Asia; while *Nitrosococcus* is a round, disk form (as the ending *coccus* implies), found in the soils of the New World. *Nitrobacter* is a short rod-shaped form that is not motile. All three forms are checked in their activity by the presence of organic compounds and, in pure cultures, are grown on a purely inorganic medium. This peculiarity in their physiology was discussed in the preceding chapter and, since organic materials act like poisons to them, it is obvious that they cannot produce nitrates from organic compounds but only from the nitrogen after it has reached the stage of ammonia.

The conditions necessary for good nitrification are good aëration of the soil, a medium water supply, a soil temperature of 30° C. with a maximum of not more than 40°, the presence of some basic compound such as calcium carbonate, and only small amounts of organic matter. It will be observed that these are exactly the proper conditions of good tillage for the majority of field crops. When the soil is in good condition for the roots of the growing crop, it is also in proper condition for good nitrification. The requirements for nitrification and fertility are nearly identical.

Ammonification.—The production of ammonia from the organic matter in the soil is the work of various organisms among which are *Bacillus mycoides*, *B. ramosus*, and *B. vulgaris*. All these bring about the decomposition of organic bodies, producing ammonia along with the other by-products of their activity. They are saprophytes and, in fact, ammonia is doubtless a by-product of the activity of most saprophytes. Omelianski (1899) has thus shown that to produce nitrates from organic matter all three groups of bacteria must be present, viz., the ammonifying, those which produce nitrites, and those which produce nitrates. If we call these A, B, and C, then, as shown in the table below, when only A and B are present the action goes no further than nitrites. If A and C are present, only ammonia is produced; while if only B and C are in the nutrient solution, there is no change of the organic material whatever. All three must be present to get any nitrates produced.



Denitrification.—While the previously described processes increase the amount of available nitrates in the soil, there are unfortunately other organisms which cause the reverse process to take place. This antithetic process is called denitrification and consists in the reduction of nitrites and nitrates with the formation of free nitrogen and its resultant loss to the soil. Denitrification is accomplished by a large number of organisms which normally live aërobically and get their oxygen from the air, but which, when forced to live under anaërobic conditions, obtain their oxygen from these nitrogen compounds that contain it. It is evident that the conditions for the maximum activity of these organisms are the reverse of those for nitrification,—poor aëration of the soil, too much water, low soil temperature, and an acid soil with much organic matter. These are just the reverse conditions for good fertility, and might be found after saturating the soil with water following a heavy application of manure. In this case, although adding organic matter for fertilizer, one is actually diminishing the amount of nitrogen and producing exactly the opposite results from those desired.

Nitrogen Fixation.—As we have seen, nitrogen is being constantly lost to the air through the processes of denitrification, and much nitrogen is also leached out of the soil and carried down to the ocean. If there were no way of tapping the vast reserves in the atmosphere, which is 80% nitrogen, the available supply of this element would ultimately be lost forever from the organic cycle once it had escaped to the atmosphere in the free form. As long as the nitrogen is in the form of nitrates or ammonium compounds, it is still in the organic cycle, because these compounds may be used by plants or may be easily put into an available form. The denitrification processes and the burning of organic compounds are the most serious causes of loss of organic nitrogen, since in both these cases the nitrogen is freed as nitrogen gas or as a gaseous nitrogen compound. The decomposition of organisms in the soil also results in the escape of some nitrogen.

To counteract this gradual loss of the nitrogen from the organic cycle, natural processes have been sought which lead to the conversion of free nitrogen into some compound easily used by plants. Three such fixation processes have been determined to date. Cavendish pointed out that an electric spark effects the union of nitrogen and oxygen, with the result that during thunderstorms the amount of nitrate is somewhat increased; Berthelot showed that the silent discharge of electricity might bring about similar *fixations* of nitrogen with organic compounds; and Schönbein has called attention to the previously mentioned fact that during evaporation small amounts of nitrogen are combined with hydrogen to produce ammonium nitrate. Of these three, only the first can be considered as of any real significance, the importance of which can be realized from the figures of Arrhenius, who has estimated that rainfall brings to earth each year one and a half billion tons of nitrogen in the form of ammonia and nitric acid. This means about five pounds per acre per year.

Man in the laboratory has tried by means of the electric spark to duplicate the fixations found in nature. One method has been to bring about the union of nitrogen with oxygen, forming nitrates. Another has been to unite the nitrogen with calcium carbide, (CaC_2) forming cyanimide; while a third has been the attempt to unite nitrogen with hydrogen, forming ammonium compounds. Recently there have been discovered catalyzers which will bring about these unions without the expenditure of such enormous amounts of energy as were formerly required. One such compound made in this manner is sodium cyanide ($NaCN$), which is formed from sodium carbonate, nitrogen, and carbon in the presence of finely divided iron as a catalyst:

$$Na_2CO_3 + N_2 + 4C = 2NaCN + 3CO.$$

These catalytic methods will put the manufacture of nitrogen compounds into the hands of countries where large water-power sources are not available, and will thus free those countries which lack water power and large deposits of nitrogen compounds from the control of their more fortunate neighbors.

Fixation by Bacteria.—What man has been striving so hard to accomplish and what inorganic nature is doing to a small extent, many of the lower plants are able to do quite successfully.

Lawes and Gilbert (1891) showed that legumes had the ability, when grown on a soil in which the nitrogen had reached its minimum, to increase the amount of total nitrogen present, while, in the case of grain grown upon a similar soil, no increase in nitrogen took place. Wagner (1891) also showed that peas were not able to profit from fertilizers containing nitrogen to the same extent as oats. These results differ from those of Boussingault, who found that all plants were equally dependent upon fertilizer, but when it is remembered that Boussingault used sterilized soil while the others did not, the discrepancy is explained.

Hellriegel and Willfarth (1888), whose researches in this field are classic, showed that nitrogen was increased only in unsterilized soil even in the case of legumes. They noticed further that the addition of nitrogen was associated with the formation of small nodules or tubercles on the roots of the legumes, and proposed that these growths were the result of a symbiotic relationship between soil microörganisms and the roots of the plant. These results have been confirmed many times, and in that same year Beijerinck isolated the bacteria concerned and grew them in pure cultures.

These bacteria (*Bacterium*, *Rhizobium*, or *Pseudomonas radicicola*) develop only on unsterilized soil and are of many different strains or races, each of which grows best in association with a particular legume. Thus nodules will form on pea roots better if soil is taken from a field where peas have been grown previously than when the peas are inoculated with soil from a clover field. There is even a difference between the race which grows on alfalfa and the one on clover. Formerly the inoculation was made by importing soil from a place where the crop was previously grown. Now the experiment stations keep on hand the various strains in pure cultures, and these are furnished to the farmers throughout the State who wish to inoculate a particular crop growing in soil deficient in the necessary strain.

The bacteria enter the root through the root hairs which they surround and finally penetrate, passing into the cells of the cortex as a bacterial filament. Having well entered the cortex, the filament branches rapidly, while the parenchyma cortex cells in its neighborhood divide actively. The result is a swelling (the tubercle) on the side of the root. The central part of the nodule contains the bacterial cells, which are now surrounded by the root parenchyma. The whole tubercle is covered with a corky layer and is connected by vascular strands to the root. The sheath which surrounds the bacterial filament later disintegrates, and the bacteria are thus freed inside the cells, where they enlarge and form peculiar Y-, V-, and T- shaped structures known as *bacteroids.* This bacteroid tissue becomes depleted of its contents, which are then used by the plant, after which the cells form *cysts* or colonies of spores. These then become disseminated in the soil after the destruction of the tubercle and are now capable of infecting other roots.

The bacteria have the power of fixing nitrogen in the form of organic nitrogen compounds from the free nitrogen in the air in the soil. The host plant is then able to absorb and use this organic nitrogen or some similar compound resulting from its partial decomposition. In just what manner the legume takes the nitrogen from the bacteria is not known, but the relation between the two organisms is apparently one of mutualism. The bacteria get their carbon foods, minerals, and water from the legume and after their death give to it in return the manufactured nitrogen compounds. The bacteria certainly profit from the relationship, and there can be little doubt of a similar advantage gained by the legume from the presence of the bacteria. When the plants are cut and the roots left in the soil, the crops which come after are able to use the excess bound nitrogen which has accumulated in the soil thus enriched by the leguminous plants.

For a long time it was believed that the legumes were the only plants associated with tubercle-forming, nitrogen-fixing bacteria, but recent workers have found many other plants which derive some benefit from similar associations. Miss Spratt (1919) reports nodules in the families of the Eleagnaceæ, Myricaceæ, Cycadaceæ, Podocarpaceæ, and in the genera, *Alnus* and *Ceanothus*. In these plants the bacteria are associated with the roots as in the legumes; but in the *leaves* of certain tropical Rubiaceæ have been found tubercles containing bacteria which are able to fix nitrogen from the air in the same manner as those which inhabit roots. The natives use the leaves of these plants as green manure. Leaves of *Dioscorea macroura* also have been found to contain nitrogen-fixing bacteria.

Nitrogen Fixation in the Soil.—Not all nitrogen-fixing bacteria are mutualistic and associated symbiotically with higher plants. Berthelot (1885) showed that the nitrogen of the soil was increased by means of bacteria living *free* in the soil, but the determination and isolation of these forms were left for later workers. Two forms in particular have been much studied,—*Clostridium* and *Azotobacter*. Winogradski (1893), working with the former genus, has shown that it is an anaërobic form and that small quantities of ammonium salts aid in the fixation process. Beijerinck (1901) later isolated the other nitrogen-fixing bacterium (*Azotobacter*) and found it capable of fixing nitrogen in the presence of oxygen.

Other bacteria and fungi have been reported as capable of fixing free nitrogen in the soil. Among these are *Phoma betæ*, a fungus which grows parasitically upon sugar beets, and several species of *Penecillium* and *Aspergillus*. Wann (1921) reported several species of green algæ as possessing this power; and Lipman and Taylor (1924) announced that wheat and barley can fix free nitrogen.

Page and others failed to confirm the work of Wann, and the technique of Lipman and Taylor has also been found not above reproach; for the present, therefore, these results are viewed as decidedly heterodox! On the other hand, there is little doubt but that blue-green algæ, such as *Anabæna*, can fix free nitrogen; and in the previous chapter mention was made of the symbiosis between this form and *Azolla*. *Anabæna*, however, is not at all dependent upon the *Azolla*, and, according to Allison and Morris (1930), who it may be added found *no* fixation with any *green* algæ, *Anabæna* can fix 5 mg. of nitrogen per 100 c. c. of culture medium in 75 days. These workers believe also that in some agricultural soils, especially rich in blue-green algæ, these may be the most important agents of nitrogen fixation.

The possible relation of mycorrhiza to nitrogen fixation has also been mentioned. Various mycorrhiza have been reported as nitrogen fixers, and Melin (1922) found that *Pinus sylvestris* could be grown in a nutrient solution completely lacking in nitrogen if inoculated with its mycorrhizal fungus. Thus the fixation of nitrogen by fungi (including mycorrhiza and bacteria) seems to be a very common occurrence, and the sum total of the nitrogen fixed by all these methods is of considerable economic importance. It has been estimated (Arrhenius) that all the organisms working in the soil may fix per year about 25 pounds of nitrogen per acre on the average; but a good crop of legumes may add eight times that much!

The Nitrogen Cycle.—The previous pages have showed the importance of nitrogen in plant nutrition and the various ways in which the nitrogen is maintained in the organic cycle, as well as the methods by which the great reserves of free nitrogen in the air may be drawn upon. Nitrogen is one of the most expensive of the elements in our food supply. It is one of the elements in which soils are commonly lacking and, for this reason, is generally present in commercial fertilizers. The necessity for the conservation of our nitrogen resources cannot be overemphasized. The nitrogen passes from the soil into plants, from there into animals, and back again to the soil. Any retardation of the action of natural forces in restoring the organic nitrogen to the inorganic form, where it can be used again by plants, withdraws that much nitrogen from circulation and is to be deprecated. Enormous quantities of nitrogenous material are wasted in sewage in America, while in France and many other sections of Europe the sewage from large cities is placed on the truck farms of the vicinity, the molecules of nitrogen are taken up by the growing foodstuffs, and within three months they are continuing their interesting and variable careers as animal foods.

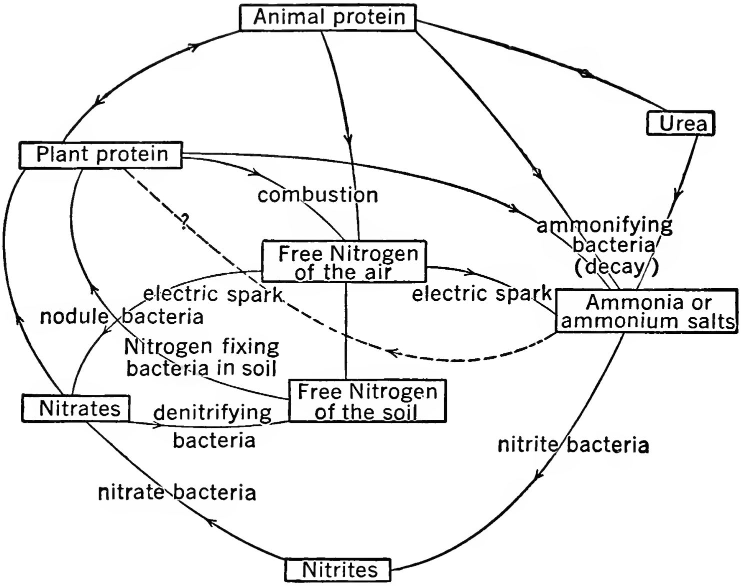


Fig. 6.—The nitrogen cycle. Arrows indicate the direction of the change.

Figure 6, which shows the various phases of the nitrogen cycle, may serve as a pictorial summary of its manifold features.

QUESTIONS

1. Under what conditions may the addition of humus be injurious to plants?

2. What are the elements generally contained in commercial fertilizers? Why?

3. An average crop of wheat removes about 50 pounds of nitrogen per acre from the soil. If nitrogen is 80% of the atmosphere by weight, how many years would the nitrogen in the air last if it were available?

4. At what point in the nitrogen cycle is loss of available nitrogen most likely to occur? Why?

5. Why does barnyard manure lose much of its fertilizing value when left exposed to the air?

6. Does old manure have the same value as fresh manure? Explain.

7. Are there any dangers from the direct use of sewage as fertilizer?

8. What is a catalyst?

9. What is the Haber process of nitrogen fixation?

10. Do soils ever lose any nitrogen aërobically?

11. Why were the farmers of the southern United States so interested in the Muscle Shoals power project?

12. Is cremation or burial more wasteful from the point of view of the nitrogen cycle?

13. Does the abundant use of nitrogen on crops ever result in any disadvantages?

14. Can *Rhizobium radicicola* free in the soil fix nitrogen or must it be in the nodules of some host plant? See *Soil Science*, 29:37, 1930.

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CHAPTER X

NUTRITIVE AND STIMULATIVE FUNCTIONS OF SALTS

> Out of the dead, cold ashes life again.
> —J. B. Tabb.

The Ash.—It has been pointed out in Chapter III that a plant can be divided into three portions, viz., a watery portion varying from 40 to 95%, a combustible portion ranging from 1 to 55%, and an incombustible residue of ash of from 1 to 5%. The water may be driven out by heating in an oven at a temperature of 105° C.; when the plant no longer loses weight at this temperature, it can be assumed that all the water has been driven off. To eliminate the combustible material which makes up the bulk of the dry matter, the plant must be incinerated, under which condition the carbohydrates, fats, proteins, etc., are oxidized and pass off into the atmosphere as gases. The carbon, oxygen, hydrogen, and nitrogen of the plant are thus driven off; and if the residue is then analyzed, it will be found to consist chiefly of oxides of the various minerals called the *ash*.

These ash elements function in the plant for the most part in one of three ways. (1) They may enter into the composition of the plant substance, in which case they are said to be *nutritive.* (2) They may cause by their presence certain processes and reactions to take place, which would not occur in their absence. In this respect they act either like enzymes (Chap. XVI) or vitamins (Chap. XVII), and for this general type of function, we may use the term *stimulative.* Since it is frequently difficult to separate these two functions, they are treated together in this chapter. (3) The substances may counteract the effects produced by some other element present. This *balancing* function, due, in general, to the fact that electrical charges come into play when matter in an ionic state is concerned, will be considered in the next chapter.

A total of over thirty elements have been found in plant ash including the following:

aluminum
arsenic
barium
boron
bromine
calcium
chlorine
cobalt
copper
fluorine
iodine
iron
lead
lithium
magnesium
manganese
mercury
nickel
phosphorus
potassium
rubidium
selenium
silicon
silver
sodium
strontium
sulphur
thallium
tin
titanium
zinc.

Of these many elements found in the ash, only twelve are common,—aluminum, boron, calcium, chlorine, iron, magnesium, manganese, phosphorus, potassium, silicon, sodium, and sulphur. These are also among the most common elements present in the soil, so it is not strange that they should be found so often in plants. Up until the latter part of the eighteenth century, it was thought that this small percentage of ash was of minor importance in the plant and accidental to the fact that the plant was growing in a medium containing these elements. Among the first to recognize the importance of the ash materials was Lavoisier (1792), who wrote: "Plants take from the air which surrounds them, from the water, and from the *mineral kingdom* the materials necessary for their organization," and furthermore showed the importance of plants in the general cycle of the elements. De Saussure (1804) insisted that the minerals found in plants were not accidental and pointed out certain ones which were essential. These views were not popular, however, and up until 1840 it was generally believed that the chief importance of fertilizer was its help in the decomposition of humus.

The Essential Elements.—To determine exactly what minerals are necessary for a plant, the method of analysis is not sufficient. Plants may take up elements which they do not need, and such has been found to be the case. Some further check is necessary, and to settle these questions the method of culture solutions is now commonly used. Plants are grown either in water solutions of various salts or in clean sterile quartz sand to which salt solutions have been added. The effect of the absence of any element can then be determined and studied. It has been shown in this manner that of the twelve elements listed above as nearly always present, only eight are *essential* for the growth of the higher plants. These are boron, calcium, iron, magnesium, manganese, phosphorus, potassium, and sulphur. Along with carbon, hydrogen, nitrogen, and oxygen they make up the twelve essential elements for plant growth. Until the last decade, only ten elements were considered essential, but as methods of the purification of salts have improved, it has been found that the list should be expanded. Whether this list will continue to grow remains to be seen. At the present time, it seems rather that we shall find that there are differences between individual species: certain elements will be found essential for some plants but not for others.

Since some of these elements are needed in the merest traces, it may be that their absence from the list of essential elements has been due to the use of impure salts in the nutrient solution, where their presence has until recently been undiscovered; or it may develop that their action is largely a balancing one (Chap. XI) and the improvement which they cause in a solution which lacks them is balancing rather than actually nutritive.

The ten elements previously considered essential could be remembered by a mnemonic system suggested by Cyril Hopkins, formerly of the University of Illinois, who used to tell his students to think of "CHOPKNSCaFeMg," which, read as "C. Hopk'ns' Cafe, mighty good" really contained the chemical symbols of the ten elements needed by all the higher plants. Since we have twelve in the list at the present time, it is here suggested that if the reader thinks of "CHOPKNSCaFeMgBMn" and reads it as "C. Hopk'ns' Cafe, mighty good, but mnemonic," he can still remember the list without any great strain on his memory.

Calcium.—Calcium is obtained from the soil as either calcium nitrate or sulphate. It is necessary in the plant for normal root and leaf development. Plants with chlorophyll need more calcium than those without and, in fact, fungi can develop in its absence. *Monotropa* (Indian pipe), the saprophyte mentioned in Chapter VII, has less calcium than normal autotrophic plants. The cell wall contains calcium pectate and calcium is necessary for its formation. Cells formed in its absence are thus likely to be weak and easily injured, not only because of the absence of calcium in the cell walls, but also because dividing cells in plants lacking calcium contain large vacuoles, like old cells, and are thus deficient in protoplasm. The cell divisions under such circumstances are also very irregular and abnormal. Likewise root hairs do not develop so profusely in the absence of calcium. Calcium favors the digestion of starch and its translocation from one part of the plant to another; and it may be for this reason that autophytes (autotrophic plants) need more calcium than heterophytes. Many plants will grow only in a basic soil, and calcium salts are commonly added to bring about the proper soil reaction. Such plants as well as those which grow wild in calcium areas are known as *calciphiles* or *calcicoles*. Others demanding an acid soil where little calcium is present are known as *calciphobes*. Since calciphobes, like calciphiles, require lime in their nutrition but an acid soil, a better name would be *acidophile*.

Iron.—Iron occurs universally in plants in small amounts, but is more abundant in physiologically active regions such as leaves and flowers. It is added to the culture solution in very minute amounts, but seems to be necessary for every living cell. According to Warburg, iron acts as an oxygen carrier and is, therefore, essential in respiration (Chap. XXV). In its absence chlorophyll will not form, and plants without it are pale and chlorotic even when grown in the light. The first leaves which develop from the germinating grain will be green but, as the iron stored in the seed becomes exhausted, the succeeding leaves become paler and paler. If a trace of ferric chloride is then added to the nutrient solution, in two or three days all the leaves will be green. As previously suggested, it is thought that the iron aids catalytically in the formation of the pyrrol ring, which plays such a prominent part in the chemical constitution of chlorophyll. In the case of chlorosis of fruit trees like the pear, a similar transformation is quickly effected by boring into the sap wood and inserting a small crystal of ferric sulphate; and spraying chlorotic trees of *Pinus banksiana* with $FeSO_4$ has been found to reduce the chlorosis from 75 to 5%. Most soils contain sufficient iron for the crops grown upon them, but in Hawaii and Porto Rico pineapples can grow only when iron sulphate is added to them. This is commonly done by spraying during growth.

Magnesium.—This element enters into the chemical composition of the chlorophyll molecule and is hence necessary for its formation; in its absence, also, chlorosis results. It is more abundant in parts undergoing development and is hence thought to be necessary for the formation of nucleoproteins (the proteins of nuclei), which may explain why nodules in leguminous plants do not develop well in its absence. Magnesium is also found abundantly in seeds and in those parts rich in fat. In *Vaucheria*, fat globules were not found unless magnesium was present. It also seems to be necessary for the transportation of phosphorus, and, since this latter element is necessary for fat formation, perhaps the magnesium affects the formation of fats only indirectly.

Phosphorus.—This element is generally supplied to the plant as phosphate and enters into the formation of many proteins, especially those of the nucleus. When young, the presence of phosphorus stimulates healthy root growth, and when the plant is mature the presence of phosphorus hastens the ripening process. It is stored in seeds, as evidenced by the common expression that "phosphorus makes seeds." Photosynthesis occurs without it, but the breaking down of the insoluble carbohydrate into a soluble form for translocation generally occurs only in its presence. According to Turner (1929), the favorable effect upon root growth is due primarily to this favorable effect upon translocation. Loew thought it also made fats assimilable. He demonstrated that there was no cell division in the absence of phosphorus and attributed this to the absence of phospholipoids, for the formation of which the phosphorus is necessary. Phosphorus aids respiration because it is a coenzyme of zymase (Chap. XXV), and Eckerson found that when phosphorus is deficient the enzyme, reductase, which reduces nitrates, is diminished so that the plant is unable to reduce the nitrates present and manufacture proteins (Chap. XIV). Although nitrates are present they cannot be used. The result is a disintegration of the protoplasm, which is checked when phosphates are again supplied.

Potassium.—This element is needed for the formation of carbohydrates and their translocation. In its absence starch is produced in noticeably reduced quantities and no reserves are stored, possibly owing to the fact that potassium may aid in the formation of invertase, which decomposes cane sugar into glucose and fructose (Chap. XII). Plants lacking this element are incapable of synthesizing proteins properly. If potassium is absent, cell division is markedly affected; the cells elongate but fail to divide and the plants are weak and very susceptible to cold and disease. The mechanical tissue is not well developed in the stems. In the absence of potassium, plants seem unable to control respiration as do normal plants, and fruit trees consequently often present a "scorched" appearance where portions of the leaves have dried up. The "bronzing" of *Citrus* leaves under similar conditions is probably related to this. Young parts are always richer in potassium than older ones, and James has concluded that the lack of potassium causes senescence; but it is more likely that the plant removes the potassium from older regions and its absence from older regions is a result of senescence rather than a cause. Potassium salts are slightly radioactive, and their effect upon the general health and nutrition of the plant has been attributed by Stoklasa (1920) to this property.

Sulphur.—This element reaches the plant as soluble sulphates and is necessary in fairly large quantities, as it enters into the composition of proteins. Although the seed contains enough to last the plant for a long time, its absence is ultimately felt. A deficiency of sulphur results in a retardation of cell division similar to that produced by an absence of potassium. Fruiting is also hindered or suppressed. Legumes are especially sensitive to its absence, possibly because it is necessary for the development of the tubercles. Most soils contain sufficient sulphur and considerable is returned to the earth in rain, but large areas of the West must be fertilized with sulphur before alfalfa or other legume crops can be grown. It is thought by many to be important also in the respiratory processes of the cell.

Boron.—Boron is among the latest elements to be proved indispensable to plants. While there are some who do not wish to admit it to the exalted position of an *essential* element and would explain its favorable action as due to its alkaline nature (Morris, 1931) or to the fact that it injures competing organisms of the soil (Kellerman), most workers are now agreed that the boron is really used by the plant. Another cause of disagreement is the fact that it is toxic if more than 5–20 parts per million are present in the culture solution. Soy beans were improved by 2.5 mg. of boron per liter in the culture solution, but when as much as a pound per acre was added to plants in the field, the leaves were distinctly injured. Likewise 50 p.p.m. retarded wheat 40%, and 100 p.p.m. stopped growth almost completely; 3–20 p.p.m. applied as sodium or potassium borate stimulated growth up to the flowering stage. There is also a great difference in the requirements of various plants. Legumes and members of the Solanaceæ (tomatoes, potatoes, and tobacco) can use somewhat larger amounts than cereals. Boron seems to be related to the utilization of calcium. In its absence, the cells cease to divide normally, the roots develop abnormally or slightly with a poor root cap, and the nodules of legumes do not form properly, owing to a poor development of the vascular tissue leading to them. The shoots become very brittle (Johnston), the phloem of the petioles becomes disorganized, with the result that the sugars are not transported out of the leaves, and the plants (tomatoes) then develop a purplish color, probably due to the development of anthocyan (Chap. XV).

Manganese.—This element is another of the newcomers among the list of essential elements, but sufficient work has been done upon it the past ten years to convince all but the most conservative physiologists that it is really a necessary element for the growth of plants. It is found in all plants and in all regions, but especially in those portions which are physiologically active (seeds, root and stem tips, etc.). For this reason and the fact that it is needed in such minute quantities, it is thought by many to function somewhat like a vitamin (Chap. XVII). Others, however, consider that it functions more as an enzyme or catalyzer (Chap. XVI). In its absence, chlorophyll does not develop properly, and the plants become chlorotic. It seems to favor oxidation processes in the higher plants and fermentation in yeast. Its complete absence from oats causes "grey speck," but so little as 1 part in 4,000,000 will produce recovery or prevent the appearance of the disease. Above 1 p. p. m. manganese tends to become toxic. It is thus seen to be needed in extremely minute amounts, and according to McHargue plants growing in unglazed earthen pots may get all the manganese they need from that which dissolves out from the vessels into the water. Although Clark and Ely (1930) did not find this element necessary for *Lemna*, McHargue and Calfee (1932) found that it was. The place of this substance among the "big twelve" commences to be so firmly established that the burden of proof now rests upon those who wish to prove its unimportance.

Other Common Elements.—Although the eight elements mentioned are the most important ones for green plants, certain other elements are of great importance in many cases. Because an element is not essential, it does not follow that it is not beneficial. Probably most ash constituents exert either a harmful or beneficial effect upon the plant which contains them.

Aluminum.—Aluminum is very common in plant ash. In the case of the common hydrangea the color of the flowers is affected by the presence of aluminum. It had been observed by gardeners that red-flowered forms when grown on certain soils produced blue flowers and, as the result of various soil analyses and fertilizing experiments, it was determined that the blue color was produced when the soil contained soluble aluminum salts. In greenhouse practice this is now the established method of producing blue-flowered hydrangeas, and provides a striking illustration of how a nonessential element may influence plant metabolism. But iron salts may also cause *Hydrangea* flowers to become blue, and Atkins finds that they are blue in any distinctly acid soil. This effect thus seems to be a question of acidity rather than a specific mineral effect.

Although aluminum is stimulating to plants in very low concentrations it is very toxic above 15 p. p. m. Since it is only slightly soluble in soils near the neutral point, i. e., in tillable soils, it is thought by Magistad (1925) that it can seldom cause much harm. What has been called aluminum poisoning is more frequently either an acid effect or phosphorus starvation, due to the precipitation of the phosphorus as aluminum phosphate.

Some plants, e. g., *Eurya japonica* of Java, absorb aluminum in great quantities; in such an "aluminum plant," over 45% of the ash may consist of aluminum oxide.

Chlorine.—This element does not enter into the composition of many organic compounds and, although essential in animal nutrition, is not essential for plants. When grown in saline conditions, plants absorb much chlorine, which raises the osmotic pressure within the cells (Chap. XIX) and thus enables them to retain their turgescence. Chlorine applied at the rate of 20–30 pounds per acre increased the yield of tobacco 10%, but 40–60 pounds resulted in a distinct injury to the crop. Although Knop and Livingston have grown buckwheat to maturity in the absence of chlorides, Nobbe obtained contrary results and attributed this lack of development to a favorable influence of chlorides upon translocation. These results are confirmed by work done upon the mango and coconuts, where chlorides in the fertilizer gave an improved yield, and by that of Tottingham (1918), who attributes the favorable results found to the effect of the chlorides upon enzyme activity; but they are not in agreement with the observations of potato growers who find that potatoes grown in soils containing an abundant supply of chlorides show a smaller yield than where chlorides are lacking. Both chlorine and iodine have been found beneficial to sugar beets.

Silicon.—The stems of cereals are especially rich in this element, the ash of corn stems being 60% silicon. The scouring rushes (*Equisetum*) contain even higher percentages of silicon, the ash here reaching as high as 70–80% of this substance. The silicon of the grasses has been thought to play an important if not essential rôle in the support of the long slender stems, but corn has been grown for four generations without it, and experiments carried on with wheat have led some observers to the conclusion that the lodging of the grain is not due to a lack of silicon, as was formerly supposed, but to crowding and insufficient illumination. The lodging is thus the result of etiolation. Anatomical studies by Koch (1872) have shown that lodged stems have all the characteristics of etiolated ones, but it is reasonable to expect that the presence of silicon would help to counteract the weakness produced by shading. Whatever rôle silicon may play in supporting the stem, it certainly helps to protect the plant against the attacks of various plant and animal parasites. Cell walls that are impregnated with silicon are not so easily penetrated by fungal hyphæ, and grains grown in the absence of silicon are much more susceptible to the attacks of rust than those where silicon is present. Similarly, plants rich in silicon have been found capable of resisting insect attacks where those lacking it have succumbed. Lemmermann (1925) and Němec (1927) have established that silicates favor the assimilation of phosphates, and a part of their favorable effect may, therefore, be attributed to this.

Sodium.—While this element is essential for animal nutrition and is very common in plants, it is not an essential one. It may partly replace potassium but, as will be discussed in the following chapter, this replacement value probably concerns its balancing function rather than its nutritive one. To the extent that it can replace potassium, it may be considered as a substitute for it, and sodium may thus help to conserve the potassium supply.

Zinc.—Some of the less common elements such as zinc are found to have very specific effects. In the seed plants, the presence of zinc may produce certain variations of color and form known as *calamin* varieties, as an example of which may be cited a calamin variety of the pansy (*Viola tricolor*). Similarly a red pigment develops in *Bacillus bruntzii* when iron salts are present. Zinc has been reported necessary for normal growth of sunflowers, barley, buckwheat, and beans, but to settle this point more work remains to be done. Allison and Hunter (1929) reported that zinc was especially needed by peanuts on certain soils of Florida at the beginning of their growth period, but towards the later stages of their development, copper was more essential. These two elements thus seemed to complement each other. In the nutrition of the fungi, zinc plays a noticeable rôle in promoting vegetative growth and hindering the formation of spores.

Copper.—Copper has been reported a necessary element for the heather in Holland, and Felix (1927) found that copper improved certain types of muck land in western New York, where lettuce and onions will not grow normally in its absence. Lipman and Mackinney (1931) state that barley will not head properly unless 1 part of copper in 8–16 millions of the culture solution is present. At present there seems to be a distinct effort to prove that this also is an "essential" element; but the work has not been entirely convincing. Sommer (1931), for example, reported that copper in small quantities was stimulating and essential to sunflowers, flax, and tomatoes. In these experiments, the plants in the culture solutions lacking copper gave much poorer growth than those with copper. But to the culture solutions were also added aluminum, arsenic, barium, boron, chlorine, cobalt, fluorine, iodine, lithium, lead, manganese, mercury, nickel, rubidium, silicon, sodium, and tin! It seems hard to believe that the plants grew poorly because of the absence of copper. The wonder is that any grew at all! In such experiments it is extremely easy to confuse a *balancing* with a *nutritive* function.

Also part of the difficulty here may arise from the confusion between a *stimulating* and an *essential* element. As many of us know, not all things which stimulate are essential. An essential element may be defined as one whose absence from a culture solution sufficiently long results in the death of the plant and which cannot be replaced by any other element. Most of the essential elements are among the twenty most common substances of the earth's crust. Some elements commonly found in plants, as mentioned before, such as nickel, cobalt, and titanium, all of which occur in most plants in small quantities, are not essential as far as we know; but no essential element is as rare as copper. It would be rather surprising if plants, in general, were unable to develop properly without their ration of this element. For the present, therefore, copper must be considered a luxury rather than a necessity.

Fertilizers.—Of the essential plant elements, only three are likely to be deficient in soils. These are nitrogen, potassium, and phosphorus, although in a few cases, sulphur may also be lacking. (Nitrogen does not occur in the ash but since it is taken into the plant from the soil, it will be treated in the following discussion with the ash constituents.) Commercial fertilizers, consequently, generally contain compounds of nitrogen, potassium, and phosphorus, which, since they are liable to be lacking, are called the *critical* elements. Iron, magnesium, calcium, and all the other essential elements are *generally* present in sufficient quantities for all *nutritive* purposes and may be neglected, although calcium (Chap. XI) is often added to diminish the acidity of the soil, i. e., for its *balancing* action.

The kind of fertilizer used will depend upon the following variable conditions:

1. *The nature of the crop plant.* Various plants have different chemical compositions and even the different organs of the same plant have the mineral salts in different proportions. The following table gives some conception of the varying composition of plant organs (after Duggar and Molliard):

Parts in 100 Parts of Ash

	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_3	SO_3	SiO_2	Cl
Seeds									
wheat	30.38	1.20	3.16	12.58	0.55	48.43	0.66	1.10	0.23
corn	29.8	1.10	2.17	15.52	0.76	45.61	0.78	2.10	0.91
bean	46.59	1.10	3.80	7.77	0.46	34.73	1.70	0.65	1.80
Fruits									
cherry	51.90	2.20	7.50	5.50	2.00	16.00	5.10	9.00	——
apples	35.70	26.10	4.10	8.70	1.40	13.60	6.10	4.30	——
Shoots									
clover	27.25	0.80	29.26	8.32	0.57	10.66	——	6.18	——
timothy	34.69	1.83	8.05	3.24	0.83	11.80	2.80	32.17	5.20
straw (wheat)	13.65	1.38	5.76	2.46	0.61	4.81	——	67.50	——
Leaves									
spinach	16.56	35.29	11.88	6.38	3.35	10.25	6.87	4.52	6.20
beech	21.80	——	44.30	7.20	2.30	7.80	2.40	10.50	——
Roots and storage organs									
beets	53.10	8.92	6.10	7.86	1.14	12.20	4.20	2.28	4.80
potatoes	60.03	2.96	2.64	4.93	1.10	16.86	6.51	2.07	3.43

This table shows us that seeds are relatively high in magnesium and phosphorus while deficient in silicon and calcium. Fruits are rich in potassium and iron. Stems and leaves are especially rich in calcium, but stems in addition contain much silicon and leaves, much iron. Roots and storage organs are richer than other organs in their potassium content.

These differences are correlated with the amount of potash, nitrogen, and phosphorus removed from the soil, as the following table illustrates (adapted from *Ill. Ag. Exp. Sta. Bul. No. 123* and the *N. Y. Exp. Sta. Bul. No. 265*):

Crop	*Yield*	*Amount in pounds removed per acre*		
		Nitrogen	*Phosphoric acid*	*Potash*
Wheat	30 bu.	42.6	7.2	7.8
Apples	400 bu.	25.6	5.9	41.50
Timothy	2 tons	48.0	6.0	47.7
Wheat straw	1.5 tons	15.0	2.4	21.0
Potatoes	200 bu.	42.0	8.7	60.0

2. *The nature of the soil.* The following table (after Duggar) shows how soils may vary in their content of the critical elements:

Soil type	*Total N*	*Total P*	*Total K*
Gray silt loam	2,880	840	24,940
Brown silt loam	5,035	1,230	35,792
Black clay loam	7,228	1,755	33,510
Yellow silt loam	2,016	884	33,901
Brown sandy loam	3,070	850	26,700
Brown bottom loam	4,720	1,620	39,970
Sandy soil	1,440	820	30,880
Deep peat	34,880	1,960	2,930

These figures indicate the number of pounds per acre in the upper seven inches of soil, but the total amount of an element present is not a true index of its nutrient value. The salts of the essential elements must not only be present but they must be present in an *available* form. A chemical analysis is thus not a true guide to the fertility of the soil. Iron is more available in the ferric than in the ferrous condition, although it may be used in the ferrous state and even in such compounds as potassium ferrocyanide. The availability of the various forms of nitrogen has been discussed in the previous chapter. Sulphur is used only in the form of sulphates and phosphorus is more available in the phosphate form than in any other. Duggar (1923) has made some interesting nutritional studies using salts which are only very slightly soluble and which thus gradually furnish the plant with the nutrient elements. The roots of plants secrete carbonic acid and possibly other acids, which help to dissolve the mineral salts and place them in an available form. Roots also probably secrete oxidizing enzymes which assist in rendering more available the minerals of the soil.

The same species of plant will vary in its ash content with the nature of the soil. Potatoes grown on a loamy soil rich in potassium have a different ash content from those grown on a sandy soil poor in this element. An idea of these variations is shown by the following figures (after Molliard):

	CaO		SO_3	
Plant	*Limy soil*	*Sandy soil*	*Limy soil*	*Sandy soil*
Cabbage	28%	13.6%	3.6%	4.6%
Clover	43.3	29.7	3.1	3.9

Similarly potatoes grown near the seashore contained 12.6% of chlorine in the ash while in the interior, away from the coast, they were found to contain only 8%.

3. *The age of the plant and its development.* If the various organs have differing proportions of the elements, it is to be expected that in the course of the development of the entire plant, as certain organs become more active than others, the proportions of the ash constituents will change accordingly. Thus wheat needs more magnesium when young than when older and barley absorbs much more nitrogen and potassium the first half of its growth period than the last. This is illustrated also by the following table for the potato:

Date	*Total per cent*	P_2O_5	SO_3	SiO_2	Cl	K_2O	Na_2O	CaO	MgO	Fe_2O_3
July 1	3.36	14.9	6.4	2.2	3.0	56.8	4.9	5.6	4.8	1.2
July 29	2.27	13.7	8.0	2.5	4.8	62.7	1.1	2.9	5.0	0.6
Aug. 28	2.66	15.1	8.5	3.4	2.7	60.1	1.2	3.3	5.1	1.3
Oct. 2	2.68	19.7	12.4	1.5	1.8	53.1	2.6	2.9	5.9	0.6

Still more striking changes are seen in the leaves of the beech as the season advances (Palladin).

Date	*Total per cent ash*	K_2O	CaO	MgO	Fe_2O_3	P_2O_5	SiO_2
May 16	4.1	42.1	13.8	4.3	0.8	32.4	1.6
July 18	4.7	17.1	42.3	5.6	1.4	8.2	21.3
Oct. 15	7.1	7.1	50.6	4.1	1.3	5.1	30.5

In reading these figures it must be borne in mind that the *relative percentage* may decrease while the *total amount* of a constituent increases.

Courtesy Bureau of Plant Industry, U.S.D.A.

TOBACCO PLANTS SHOWING GROWTH IN ABSENCE OF ESSENTIAL NUTRIENTS

The plants are grown in nutrient solutions deficient in the following elements: 1, nitrogen; 2, phosphorus; 3, potassium; 4, calcium; 5, magnesium; 7, boron; 8, sulphur; 9, manganese; 10, iron. All these elements are present in number 6.

Total Ash Content.—If the proportions of the individual ash constituents change with age and the other above-mentioned conditions, no less can be said for the *total* ash content, the specific variation of which is shown by the following table:

Total per cent of dry weight in ash

Apples	0.4	Hops (leaves)	13.6	Scotch pine	5.9
Beets	6.0	Onions	5.2	Tobacco (leaves)	11.9
Carrots	5.4	Potatoes	4.0	Tobacco (stems)	7.7
Chicory	6.2	Rice	1.2	Wheat (seeds)	2.0

Fruits, especially fleshy fruits with a high water content, have the lowest percentage of ash, and leaves have the highest, probably owing to the fact that the water leaves the ash behind as it evaporates from them in transpiration. It is common knowledge that burning leaves give more ash than other plant parts.

The nature of the soil and the environment will also cause a variation in the total ash content. With a high salt content in the soil the total ash will be higher than when the salt content of the environment is at a minimum, although it should be added that this statement is true only for *total* ash and not for any individual salt. Sugar beet ash content has been found to vary from 3.5 to 14.5% of the dry weight, depending upon the varying environmental conditions of light, temperature, soil, and the available water.

The total ash also varies with the age of the plant. Arendt determined that for oats the total percentage of ash varied from 8.57% when three leaves had unfolded to 5.40% at maturity. The following figures (from Molliard) have been found for wheat:

Age	*Per cent ash content*
Very young stem	3.6
30 days old	7.8
15 days before blooming	8.5
In bloom	6.2
Matured	5.0

These figures must not be interpreted to mean that the *total* ash is diminished, but must be interpreted in terms of a decreasing water content as well as an increased dry weight.

Nutrient Solutions.—For experimental purposes, nutrient solutions of distilled water and the essential elements are prepared. The plants may be grown with their roots in the solution, or the solution may be poured over clean sterile quartz sand and the plants grown in this. The cultures should be kept so that the roots are in the dark, and it has been found best to keep the solution slightly acid. The seed contains much stored food and as soon as the roots have reached a sufficient length the cotyledons should be removed; otherwise there will be no visible effect of a deficient element for a week or two. The concentration of the solution must not be too strong or the roots will become plasmolyzed (Chap. XIX), and for the reasons discussed in the following chapter the salts should be in a fixed ratio which varies with the plant and the stage of its development. Various solutions for general purpose work have been prepared and found suitable for laboratory work. Among these are:

Knop's solution

Water	1,000 g.
$Ca(NO_3)_2$	1.0
KNO_3	0.25
KH_2PO_4	0.25
$MgSO_4$	0.25
$FePO_4$	trace

Detmer's solution

Water	1,000 g.
$Ca(NO_3)_2$	1.0
KCl	0.25
$MgSO_4$	0.25
KH_2PO_4	0.25
$FeCl_3$	trace

Pfeffer's solution

Water	3–7 l.
$Ca(NO_3)_2$	4 g.
KNO_3	1
$MgSO_4$	1
KH_2PO_4	1
KCl	0.5
$FeCl_3$	trace

Crone's solution

Water	2.0 l.
KNO_3	1.0 g.
$FePO_4$	0.5
$CaSO_4$	0.25
$MgSO_4$	0.25

Shive and Tottingham have worked with a three-salt solution which has proved very satisfactory. That of Shive (1915) consists of monopotassium phosphate, calcium nitrate, and magnesium sulphate with a trace of iron phosphate. It will be observed that these solutions contain no boron or manganese. These elements are needed in such small quantities that salts which have not been purified by very special processes generally contain enough of boron and manganese to prevent any of their deficiency symptoms from appearing.

Crop Rotation.—Since different plants take from the soil different materials, it would seem inadvisable to continue using the same soil for the same crop for several consecutive years. It is a common agricultural practice to grow plants in a consecutive series or to follow what is called a system of "crop rotation." If the failure of a crop is due to the withdrawal of certain essential elements, fertilizers should be able to make the rotation unnecessary. While the addition of fertilizers does enable the farmer to disregard rotation to a certain extent, the time ultimately comes when the crop deteriorates.

This has led to a second theory of crop rotation, which has been developed by the U. S. Bureau of Soils. This is known as the *toxin* theory and, according to its proponents, the failure of a crop following itself is due to the production in the soil of autotoxins or poisonous metabolic products excreted by roots into the soil. These harmful products accumulate and finally prevent the growth of the same crop although other crops are not so much affected by them and may even be stimulated by them.

Another theory of crop rotation is the *sanitation* theory whose followers insist that the cause of crop deterioration is the presence in the soil of plant diseases. These microörganisms, which are more or less specific for each crop, ultimately break down the resistance of the plant growing on any particular plot of land and cause a constant diminution in the yield. Other plants may grow on that soil, however, without being affected.

All three of these causes doubtless work together to make a rotation advisable. In such rotations some legumes should always be included because, of all crop plants, they are the only ones which ever leave the soil richer in any element than they find it.

QUESTIONS

1. Why are some plants "heavy feeders" on the soil and others "light feeders"?
2. Why should different plants use different materials from the soil?
3. If plants lack chlorophyll, what may be the cause?
4. Which requires more potash,—lettuce or wheat? more nitrogen?
5. Why are the necessary elements among the most common ones in the earth's crust?
6. What are toxins?
7. Why must the "I" be omitted in the mnemonic system for remembering the twelve essential elements?
8. Can plants use pyrrol salts in place of iron? See *Am. Jour. Bot.*, 13:276 1926.
9. Why is sulphur returned to the earth in rain? See *Plant Physiol.*, 1:77, 1926.
10. What is meant by a "4:4:3 fertilizer"?
11. Only recently has it been possible to get iron free from impurities such as manganese. Of what significance is this?
12. Johnston calculated that when a plant is nourished by one part of boron in two million of solution, the nutrient solution is as concentrated as pea soup, made up of one pea in 132 gallons of water. Was his calculation correct?

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CHAPTER XI

THE BALANCING FUNCTION OF SALTS; IONS AND ANTAGONISM

> A world . . . exists by the balance of antagonism.
> —CARLYLE.

Toxicity.—From the time of the earliest studies on nutrient solutions, it has been observed that any one of the nutrient salts may be toxic or inhibit growth when employed alone. This toxic effect is not produced by plasmolysis (Chap. XIX), but is due to some injurious action of the salt upon the protoplasm of the plant. Several observers of late years have measured this toxic effect; methods of combating it have been developed: and its cause has been explained by many theories.

Kahlenberg and True (1896) as well as Coupin have expressed the toxicity of different mineral salts in respect to wheat by a number, called the *equivalent toxicity*, which represents the minimum quantity in grams which when dissolved in 100 c. c. of water will cause the death of the plant (Coupin):

KH_2PO_4	6.0	$MnSO_4$	1.9	$MgSO_4$	0.8
NH_4NO_3	3.9	$CaCl_2$	1.85	$ZnSO_4$	0.12
KNO_3	3.0	KCl	1.9	KBr	0.10
		NaCl	1.8	LiCl	0.04

In some cases the toxicity seems to vary directly with the atomic weight of the metal, but that it does not always follow this simple scheme is shown by the fact that salts of lithium (7) are more toxic than those of sodium (23) and of potassium (39). Often the toxic effect is the additive effect of the two kinds of ions which compose it. The iodides are more toxic than the corresponding bromides, which, in turn, are more toxic than the chlorides, as the following table (after Molliard) indicates:

	Chloride	*Iodide*
Na	1.8	0.65
K	1.9	0.05
Ca	1.85	0.31
Ba	0.235	0.019
Sr	1.5	0.093
Zn	0.30	0.07

In the same way the various sulphates have an equivalent toxicity which varies with that of the metal contained:

K_2SO_4	2.3
$MnSO_4$	1.0
$ZnSO_4$	0.12
$CdSO_4$	0.025
$CuSO_4$	0.0055

From the above tables it will be noticed that, of the compounds used by plants in moderately large amounts, magnesium salts are the most toxic. These figures are supported also by the results of Kearney and Harter (1907), who find that magnesium salts in alkali soils are very toxic to wheat. Corn, on the contrary, is not harmed as much by magnesium as by sodium under these conditions. The high toxicity of boron and manganese was mentioned in the preceding chapter.

Antagonism.—It has been noticed by many workers on this subject that when salts are mixed in proper proportions, the toxic effect of the individual salts is overcome. This phenomenon was early studied by Loeb, who used the eggs of *Fundulus* (a minnow) for his experimental material. When placed in a solution of sodium chloride of the same concentration as the concentration of this salt in sea water, no development of the eggs occurred; but when a trace of calcium chloride, zinc chloride, or of some other bivalent salt was added, the development was normal. The calcium chloride or zinc chloride by themselves are also toxic. The neutralizing in this manner of the toxic effect of one salt by another, also toxic when used alone, is called *antagonism.*

Osterhout has studied this matter especially in plants, using both marine and terrestrial forms. The following are some of his results:

Plant	*Solution*	*Time before death*
Lunularia	dilute sea water	200 days
	distilled water	200 days
	NaCl	4 days
	1000 parts NaCl and 10 parts $CaCl_2$	100 days
		Growth of roots
Triticum (wheat)	NaCl	59 cm.
	$CaCl_2$	70 cm.
	1000 parts of NaCl and 10 parts $CaCl_2$	254 cm.

The plants are thus shown to grow more and live longer in mixtures of the two salts than in either one by itself, but to produce the maximum antagonism the salts must be in the proper proportions.

0.12 M NaCl	0.164 M $CaCl_2$	*Growth in mm. (wheat roots)*
0 c. c.	100 c. c.	55
25 "	75 "	105
50 "	50 "	180
75 "	25 "	298
85 "	15 "	370
95 "	5 "	435
99 "	1 "	300
100 "	0 "	55

From the above table it is seen that, to produce the best results, the two chlorides of sodium and calcium, which are equally toxic when used alone, must be mixed in the proportions of 95 parts of sodium chloride to 5 of calcium chloride.

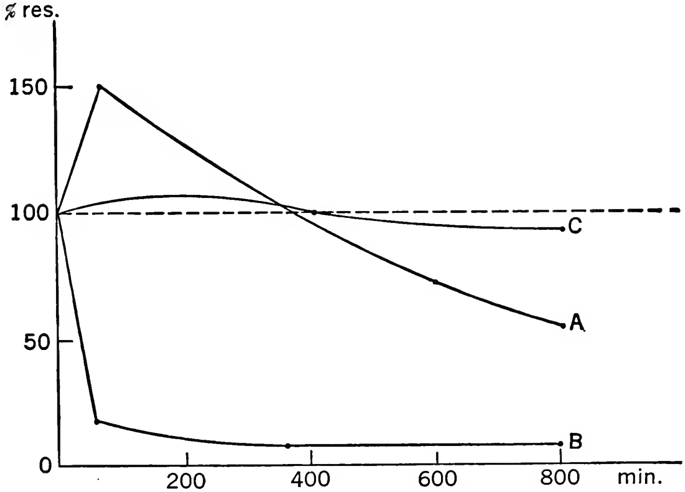

FIG. 7.—Illustrating antagonism. Curve A shows the change in resistance of *Laminaria* tissue in pure $CaCl_2$. Curve B shows the change induced by a pure solution of NaCl. Curve C indicates what happens in a solution of 97.5 parts of NaCl and 2.5 of $CaCl_2$. The dotted line indicates the condition in sea water. (After data of Osterhout.)

Working over a long period of time with many kinds of tissue, Osterhout concluded that these antagonistic effects were connected with changes which the salts produced upon the permeability of the cell. In a previous chapter, the plasma membrane has been mentioned as that outer surface of the cell protoplasm which lies next to the cell wall. Before any salt can affect the cell it must either get into the cell or else change the surface of it in such a way as to alter its permeability. Osterhout found that salts are divided into two groups: one group behaves like calcium chloride and causes the resistance of the cell to increase (or the permeability to diminish) and later diminish; while the other group, in which sodium chloride belongs, causes the resistance to decrease (or the permeability to increase) from the very start (Fig. 7).

Those which belong in the first group are the chlorides, nitrates, etc., of the bivalent and trivalent metals including $CaCl_2$, $CaNO_3$, and $MgCl_2$; while in the second group are to be found the corresponding salts of the monovalent metals such as KNO_3, KCl, $NaNO_3$, and NaCl. Acids belong in the first group and alkalies in the second. Any one of the substances in the first group as well as any one from the second will cause the death of the plant if given alone; but, when placed in the proper proportions with an electrolyte from the other group, antagonism results and the plant is not seriously injured.

An Explanation of Antagonism.—As has been pointed out by Osterhout, any theory of antagonism must explain the following points:

1. Why both sodium and calcium alone are toxic.
2. Why antagonism results in mixtures of the two.
3. Why they produce opposite effects upon the permeability of the protoplasm.
4. Why the increase in resistance produced by calcium chloride is later followed by a decrease.
5. Why all toxicity disappears in a balanced solution.

Many theories have been advanced to explain the facts of cell permeability and the phenomena of antagonism. The test of a theory is the number of facts it can explain, the number of results that can be predicted by its use, and its agreement with the facts of the related fields (in this case colloidal chemistry and biophysics). At the present time there have been collected great masses of facts, but no coherent scheme has been suggested to explain them as a unit. Such work needs badly to be done. The general nature of colloids, the behavior of salt molecules in solution (i. e., their dissociation into ions bearing electrical charges), and the properties of porous filters are sufficiently well known to provide us with a base for future operations. Accordingly Raber has proposed a theory of antagonism which seems to fulfill at least some of the requirements of a satisfactory theory in this particular field.

Attention has already been called to the fact that protoplasm is colloidal. It probably bears an electrical charge on its colloidal particles, and this charge, according to various investigators, is negative. The blood is slightly alkaline and sea water is also slightly alkaline, which facts furnish reasonable grounds for the assumption of a negative charge on the surface of protoplasm. Schaede (1924), Heilbrunn (1925), and Robbins (1926) have brought forward additional evidence in support of the negative charge on the surface of protoplasm. The cell sap on the other hand is generally slightly acid, forming with the protoplasm what is called an "electric double layer."

The second assumption in this explanation is that although a salt may be "neutral," its effect upon the protoplasm may not be, but will depend rather upon the individual effects of the ions into which the salt dissociates. In $\overset{+}{\mathrm{Na}}\overset{-}{\mathrm{Cl}}$ a univalent positive ion (the Na cation) is associated with a univalent negative ion (the Cl anion). In $\overset{++}{\mathrm{Ca}}\overset{--}{\mathrm{Cl}_2}$ the two positive charges of the bivalent cation are concentrated upon one ion while the two negative charges of the salt are divided between two anions. The dominant effect of the salt is that produced by the cation with its double charge and the $CaCl_2$ is thus a "positive" salt, as are in general all salts whose cation has a greater valence than the anion. Sodium sulphate $\left(\overset{++}{\mathrm{Na}_2}\overset{--}{\mathrm{SO}_4}\right)$ would similarly be a "negative" salt. When a plant is placed in a solution of calcium chloride the negatively charged particles of the surface of the protoplasm are at first drawn together as the "positive" salt strikes them. This causes a decrease in permeability as the membrane "tightens," but later as more positive salt diffuses in, the surface particles become positively charged and repel each other. This repulsion causes the later effect of calcium, i. e., the decrease in resistance or increase in permeability.

A "negative" salt will only add to the negative charges already present and will cause a continuous increase in permeability from the very start. A salt like NaCl in which both ions have the same valence, for reasons which cannot be taken up in an elementary text, behaves like a negative salt when placed in contact with a negative colloid. All salts can thus be divided into two groups—those that are "negative" and those that are "positive." Acids behave like positive salts owing to the very active hydrogen ion, and alkalies behave like negative salts. The size of the radical or ion also plays a part, because the charge concentrated on the surface will be more intense the smaller the ion.

Thus we see that $\mathrm{CaCl_2}$ and NaCl are toxic because both produce abnormal changes in the protoplasmic membrane, owing to the electrical charges which they possess. Antagonism results because these effects are opposite, and when the salts are mixed in the proper proportions the electrical charges are neutralized. All toxicity disappears in a balanced solution because the salts and their ions are balanced electrically.

It is noteworthy that of the six elements which plants take from the soil in any quantity, viz., N, Ca, K, Mg, S, and P, three occur in the anion with five negative charges while the three in the cation have five positive charges $\left(\overset{-}{\mathrm{NO_3}},\ \overset{--}{\mathrm{SO_4}},\ \overset{--}{\mathrm{HPO_4}},\ \overset{++}{\mathrm{Ca}},\ \overset{++}{\mathrm{Mg}},\ \text{and}\ \overset{+}{\mathrm{K}}\right)$. Iron is generally added as iron phosphate $\left(\overset{+++}{\mathrm{Fe}},\ \overset{---}{\mathrm{PO_4}}\right)$, and the two latest additions to the list of essential elements (Mn and B) are absorbed as $\overset{++}{\mathrm{Mn}}$ and $\overset{--}{\mathrm{B_4O_7}}$. While these facts do not prove anything, they can hardly be mere coincidences.

The Soil Salts; Base Exchange.—In the cultivation of plants, fertilizers containing various plant nutrients, as discussed in the previous chapter, are added to the soil. But the soil is an extremely complex mixture of microörganisms, decaying organic matter, and fine particles of disintegrating rocks, ranging in size from stones or pebbles down to particles of colloidal size and even to molecules and ions in solution. The result is that physicochemical reactions that occur between the various particles of the soil (not counting the biological activities) are so varied and interrelated that it is difficult to know exactly what salts are reaching the plant even when one knows what has been added to the soil. Colloids of the soil absorb on their surfaces some of the salts added, and in some cases one ion of a salt may be absorbed more than the other. Likewise the plants may absorb one ion more than another. This differential adsorption and absorption of ions, e. g., cations, may then leave the remaining anions free to replace anions in other salts, thus ultimately releasing in the soil solution anions that are different from those originally added to the soil.

If cations are taken up by the soil colloids and other mineral cations or *bases* are freed into the solution, this exchange of basic ions is commonly spoken of as *base exchange.* The fertility of the soil depends to a large extent, for reasons that should be already clear, upon the chief, available bases in the soil. If sodium or magnesium ions become too available, the soil becomes too alkaline and toxic to many plants. If, as often happens, the hydrogen ion becomes predominant or in excess, the soil will be acid.

Hydrogen Ions.—This brings us to one of the most interesting phases of plant nutrition. As we have seen, hydrogen is not more important in plant nutrition than carbon or oxygen; but hydrogen is one of the ions formed by the dissociation of water, the "universal" solvent, around the properties of which life itself is organized. Furthermore, hydrogen is one of the ions formed when acids dissociate; and the previous sections have shown us the importance of this active little ion in the problems of antagonism.

All acids, alkalis, and salts when in solution, as well as water itself, dissociate into cations (with + electrical charges) and anions (with − charges). A "neutral" solution is not a solution with no acid (H) ions or alkali (OH) ions, but rather one in which the number of acid ions is balanced by the number of alkaline ones. When water dissociates, it does so according to the "law of mass action" which "governs" such reactions:

$$\frac{[\mathrm{H}^+]\times[\mathrm{OH}^-]}{[\mathrm{H_2O}]}=\mathrm{K}.$$

This means that the product of the numbers of H^+ and OH^- ions divided by the number of undissociated molecules is a constant. Pure water dissociates to an extremely small degree, which means that the numerator of this fraction will be very small and the denominator very large; the result is that K will be very small. In fact it has been found by experimental methods that in the case of pure water K is 10^{-14} g. molecules per liter. Since water is neutral, the number of H and OH ions must be equal, and therefore the H ions in pure water (or a neutral solution) is 10^{-7} g. mol. per liter. This is another way of saying that pure water is 1/10,000,000 Normal in respect to the H and the OH ions. Now instead of saying that a neutral solution contains 10^{-7} g. mol. of hydrogen ions, which would be a long and inconvenient way of stating the matter, we say that water "has a pH of" 7.

This type of equation applies equally well to all solutions of acids and bases, and it is possible in all cases to express the *active* acidity by an appropriate pH number. It will be seen that if there were more hydrogen ions present in the solution, the number of hydroxyl (OH) ions would decrease, since K is a constant. When a number with a negative exponent *increases*, the *numerical value* of the exponent decreases (e. g., 10^{-7} is less than 10^{-5}). When we express the active acidity by the "pH value," we are using this numerical value of the hydrogen ion exponent; therefore, the *smaller* the pH value, the *larger* the number of hydrogen ions and the greater the active acidity.

Also it will be seen that when we know how many H ions there are in a solution, we also know how many OH ions are present, since their product is a constant. For this reason, the expression of the pH value is extremely convenient. The reader should also be able to see that a solution of pH 4 has 10 times as many H ions as one of pH 5 ($1/10^2$ is how many times $1/10^3$?).

With these chemical preliminaries out of the way, we are now ready to proceed with our physiology. The development of most species of plants is possible only within rather narrow limits of H^+ ion concentration. Soils generally have a pH value between 3 and 11, but the former would be very acid and the latter very alkaline. Some plants prefer soils of a certain pH value and others of another. In fact, in buying a farm or judging the value of a soil, the nature of the plants growing thereon furnishes a very valuable guide and a fairly reliable index to the present cultural value of the soil as far as the acidity is concerned.

By far the vast majority of plants thrive best near the neutral point. Alfalfa, clover, lupines, rye, spinach, and wheat grow best at pH 6.0–7.5; buckwheat, corn, oats, strawberries, onions, potatoes, soy beans, timothy, and turnips can thrive on somewhat more acid soils at pH 5.0–6.5; while barley, sugar beets, and tobacco thrive best on soils somewhat alkaline (pH 7.0–8.0). These are all seen to be associated with soils not extremely far from neutral, but coffee is said to require soils as acid as pH 4.0–5.0, and pineapples grow best at pH 4.0–6.0.

Plants are often very specific in their pH requirements, and two species of the same genus may have very different demands. *Galium sylvester*, e. g., requires neutral or slightly alkaline soil, while *G. saxatile* grows best on acid soils. Many similar examples could be cited. Seed germination and growth have an optimum pH value, and there is hardly a living process for which there is not an optimum H^+ ion concentration. Also different organs of the same plant may show differences in pH value; thus Gustafson found in corn, sunflower, and pumpkin that the H^+ concentration increased from base to tip. During the last twenty years papers by the thousands have been published on the minimum, optimum, and maximum pH values of the various life processes and phenomena, and this "fad" has reached such heights that it has been proposed to call the doctor's degree in physiology a pH D! From this mass of data, however, many valuable and practical results have been obtained.

Changes in soil pH may influence the plant (a) either by directly affecting the protoplasm of the root cells and the cell processes therein, or (b) by changing the relations of the various materials present in the soil. The change in cell permeability is an example of the first kind of effect, while the effects upon base exchange would be an example of the second. The effect of acidity upon the solubility of aluminum is another instance of the indirect effects of acids. When a substance is added to the soil, it is often difficult to know, therefore, whether the plant has been directly or indirectly affected.

Differences in the absorption rate of the two ions of a salt may make a great change in the pH value of the solution. This point has been mentioned in the discussion of nitrate *vs.* ammonia nutrition of plants (Chap. IX). If bases are left in the soil, it will tend to become alkaline (as when nitrates are used); while if acid groups are left, the soil, of course, will become more acid (as when ammonium salts are used). In general, plants tend to absorb ions so that the result is to bring the soil reaction nearer to neutrality. This action is further favored in certain cases by the active secretion of substances by the roots. Thus if a plant were grown in an acid soil, the soil reaction in the vicinity of the plant could be made more nearly neutral by absorbing the acid radicles from the soil or by actively excreting alkaline substances. Jacobson found that wheat plants 100 days old changed the reaction of a culture solution in 12 hrs. from pH 3.9 to 6.3, due possibly to a larger absorption of NO_3. Plants, in general, tend to bring the reaction of the solution to pH 5.0–7.0 when in solutions more acid or alkaline than this. Rice, however, formed an exception (Jacobson) in that it changed the reaction from pH 5.0 to pH 3.0 in three days. This was attributed to (a) less absorption of sulphates than of cations and (b) the excretion of carbonic acid by the roots.

On the other hand, the original pH of the soil solution determines to some degree what ions are absorbed. Thus Hoagland found that absorption of NO_3, Ca, and PO_4 was greater at pH 5.0–5.5 than in neutral soils by crops that thrive best at these pH values. There is, consequently, a mutual interaction between the plant and the soil, the details of which are not yet sufficiently explored and correlated.

Plants and animals contain also an *internal* mechanism which permits them to control to some extent their internal pH value. This consists of the action of the ampholyte and "buffer" systems of the organism. By an ampholyte is meant *organic* compounds (proteins, lecithins, amino acids), which can split off either alkali or acid groups to suit the occasion and thus neutralize the harmful effect of incoming H and OH ions. The "buffer" systems are *inorganic* salts which serve to regulate the dissociation of their acids and thus serve as potential reserves of anions and cations.

Active, Titrable, and Potential Acidity.—In addition to the *active* acidity, which is measured in terms of pH value, mention should be made of the *total* (or titrable) acidity, which refers to the acids present that are not dissociated. It must be borne in mind that "active" acidity refers only to the H ions that are active in the solution. If these are removed, then more may appear until the acid present is completely dissociated. These undissociated acids also may influence crop production, although they are of more interest when they are dissociated or active. *Potential* acidity refers to the aluminum, iron, and other compounds of the soil, which under certain conditions might be replaced or exchanged and thus free H ions into the soil. The relation of this type of acidity to plant growth has been only slightly studied.

Conclusion.—Plants originated in the sea and were developed under an environment containing a certain quantity of salts, which were in a definite ratio to each other. As the years have passed the total *quantities* have increased but the *ratios* have remained fairly constant. Similarly the *ratios* of the various salts in the blood to each other are approximately the same as those of the salts in the sea; the *concentration* in the blood is much less than that in the ocean, and probably represents the concentration in the sea in the far-away Cambrian period when animal life first developed on the land.

These salts in plants fulfill three different functions. (1) Some are used in the chemical composition of the plant body and its products. To that extent they are *nutritive.* (2) Some are probably *catalytic* in their action and, while they do not enter into the final products, they cause these reactions to come to pass. (3) Other salts are purely *balancing* in their action. They antagonize the harmful effects produced by others and furnish a medium of the proper acidity and hydrogen ion concentration for the chemical reactions necessary for optimum growth and development. In its nutritive effect a salt *cannot* be replaced by any other one. In its catalytic effect there is evidence that it *may* be so replaced, while in its balancing effect there is no doubt but that it *can* be replaced by a number of other salts. This explains why potassium can be replaced *partly* by sodium but not entirely. Sodium can take the place of potassium as an antagonizer of the harmful effects of calcium but it cannot replace potassium in its beneficial, nutrient, chemical reactions. It may also develop that the toxicity of magnesium chloride is due partly to its electrical charge and partly to some other effect; the former is antagonized by the sodium and the potassium chlorides while the latter is antagonized by the calcium chloride.

As mentioned in the preceding chapter it is often difficult to distinguish a balancing from a nutrient effect. Thus it has been noted that if fruit trees are lacking in potassium, brown patches appear upon the leaves, which are followed by premature leaf fall. This seems to be a nutrient effect, but if there is too much nitrogen in the supply of nutrients, the addition of potassium does not help. There is thus seen to be some more complex difficulty than the mere absence of potassium as a nutrient.

Theoretically, there should be some *best* concentration of these various elements for each plant, but practically this has been found very difficult to ascertain, owing to the effect of the various factors such as age of plant, time of year, other elements present in the soil, etc. Physiological balance is thus seen to be such a complex matter that comparatively few results which can be applied by the farmer have yet been obtained.

QUESTIONS

1. What is meant by permeability?
2. In what part of the cell do changes in permeability occur?
3. What is an electrostatic theory?
4. Which is more important in balanced solutions—the concentration of the salts or their ratio to one another?
5. Are acid solutions rich in positive or negative charges? Why?
6. What is the difference between total acidity and hydrogen ion concentration?
7. When the pH value increases, does the $\overset{+}{H}$ ion concentration decrease or increase?
8. At pH 7 how does the number of $\overset{+}{H}$ ions in a solution compare with the number at pH 8?
9. What is meant by the statement that the pH value varies inversely with the $\overset{+}{H}$ ion concentration?
10. Which is more actively acid, a solution of pH 8 or one of pH 10?
11. What is the meaning of the "p" in "pH"? Why not "nH"?

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CHAPTER XII

THE CARBOHYDRATES

> The two noblest things . . . are sweetness and light.
> —SWIFT.

The carbohydrates make up the bulk of the dry matter of the plant. They are the first products of photosynthesis, and it is to be expected that they will play a fundamental part in the nutrition and life of the plant. The word implies that carbohydrates are composed of carbon and water, i. e., they contain carbon, hydrogen, and oxygen, the latter two elements in the same proportions as in water. Not all, however, have twice as many hydrogen as oxygen atoms: rhamnose has the formula $C_6H_{12}O_5$. Acetic acid, on the other hand, with the formula $C_2H_4O_2$ is *not* a carbohydrate, even though the hydrogen and oxygen atoms are in the same proportion as in water.

The carbohydrates are mostly open chain compounds, with the carbon atoms linked together in the manner of compounds of the aliphatic series. From the point of view of the chemist they may also be considered as aldehyde or ketone derivatives of alcohols. (Compare the formula of glucose which is an aldehyde with fructose which is a ketone.)

Use of Carbohydrates.—The carbohydrates are widely distributed in plants and, as mentioned above, make up the bulk of their dry matter. They have in general five functions:

1. They are used in building up the cell walls. Cellulose is a carbohydrate which composes the bulk of certain tissues such as wood and straw. The carbon burned in wood is the carbon of the carbohydrate and comes mostly from cell walls.

2. They supply energy. Sugars and starches are the chief energy producers of the plant and make up the greater part of the food placed in special storage organs, such as roots tubers, and many seeds. They are also the basis for the construction of fats and proteins, which are the other food sources of energy and building materials.

3. They hold water, and in many desert plants, in the form of the pentosans, play a prominent rôle in conserving a scanty water supply.

4. They increase the osmotic concentration of the cell sap and aid in maintaining the turgidity of the plant. The soluble sugars thus aid in regulating the amount of water which enters or leaves a cell.

5. They aid in the formation of pigments. The red and blue coloring matters seem to depend upon the presence of carbohydrates for their formation, but this will be discussed further in the chapter on pigments.

Classification of Carbohydrates.—The carbohydrates are divided into two groups,—the sugars and the nonsugars. The former have a sweetish taste, which varies in degree according to the sugar. They are given names ending in *ose*, and wherever in organic chemistry such a name is found, one can be sure he is dealing with a carbohydrate.

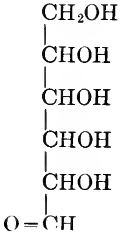
I. Sugars.
  1. monosaccharides.
    a. pentoses.
    b. hexoses.
  2. disaccharides.
  3. trisaccharides.
  4. tetrasaccharides.

II. Nonsugars—polysaccharides.
  1. food materials—hexosans.
  2. structure materials.
    a. gums.
      1. natural gums and pentosans.
      2. mucilages and pectins.
    b. celluloses.

Sugars.—Among the best known hexoses of plants are fructose, glucose, mannose, galactose, and sorbose, all of which have the formula $C_6H_{12}O_6$. Many others have been prepared with the same formula and still others are theoretically possible. How can one have several compounds with the same formula? Different substances having the same *empirical formula* or ratio of different atoms in the molecule are called *isomers*, and this property of forming isomers is one of the striking characteristics of the sugars. These isomers are of two different types,—*structural* and *stereo*isomers.

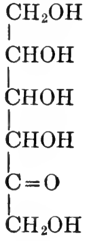
Structural isomers may be illustrated by the formulæ commonly given for glucose and fructose:

Glucose



$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ O{=}CH \end{array}$$

Fructose

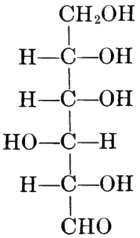


$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ C{=}O \\ | \\ CH_2OH \end{array}$$

The difference lies in the *location* of the atoms in the chain. In glucose, the oxygen not associated with a hydroxyl (OH) group is on the end carbon atom, while in fructose it is on the one next to the end, which is the difference between the aldehyde and the ketone mentioned above. Similarly the oxygen could occur in other places, producing in each case a different sugar.

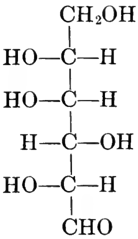
Stereoisomers may be illustrated by d-glucose and l-glucose:

d-glucose



$$\begin{array}{c} CH_2OH \\ | \\ H-C-OH \\ | \\ H-C-OH \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ CHO \end{array}$$

l-glucose



$$\begin{array}{c} CH_2OH \\ | \\ HO-C-H \\ | \\ HO-C-H \\ | \\ H-C-OH \\ | \\ HO-C-H \\ | \\ CHO \end{array}$$

Here it will be seen that l-glucose has the same kind of atoms on each carbon atom in the chain, but the *arrangement* is such that one is the mirror image of the other. With both stereo and structural isomerism possible, it is easy to see how so many different sugars with the same formula are obtained.

Rotatory Power.—The carbohydrates which are soluble will rotate the plane of polarized light passed through the solution. The degree of the rotation is measured by a *polarimeter* (an instrument of the physical chemist) and depends upon the kind of carbohydrate, the concentration, and the length of the column of solution through which the beam of polarized light has to pass. But the same length of the same concentration of the same sugar at the same temperature will always produce the same effect, i. e., the sugar has a *specific* rotatory power. Sugars which rotate the beam to the right are called *dextro*rotary and those which rotate it to the left are called *levo*rotary.

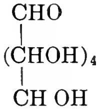
Reducing Action.—Because of the aldehyde or ketone group, the monosaccharides and some other sugars are reducing agents. The test for sugar with Fehling's solution, a standard solution of copper sulphate, depends upon this reducing action, the solution being reduced by the sugar to cuprous oxide. The amount of the oxide produced bears a definite relation to the amount of sugar present.

Formation of Glucosides.—It is possible to replace one of the hydrogen atoms of the sugar molecule by compounds of many different types thus producing a compound of a sugar with a substance entirely different. These compounds, which are called *glucosides,* can in turn be broken down into the sugar and the other radical. When hydrolyzed they are thus broken into glucose and the hydroxyl of the accompanying substance. Although strictly speaking the glucosides are compounds which yield only glucose, the term is often used more loosely to include such compounds of other sugars as well.

Osazones.—Owing to the presence of the aldehyde or ketone group in the molecule, the sugars are able to form with phenylhydrazine ($C_6H_8N_2$) characteristic products known as hydrazones and osazones. The former are mostly colorless compounds soluble in water and do not help much in the identification of sugars, but the osazones, in which an extra molecule of phenylhydrazine is added to the sugar, are not soluble in water and give crystals of a specific melting point and characteristic shape, which are very useful in identifying sugars.

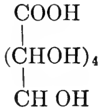
Formation of Acids.—When the sugars are oxidized they give rise to three different types of acids varying with the conditions of the process and the type of oxidizing agent employed. This may be illustrated by glucose, which when oxidized gives gluconic, glucuronic, or saccharic acid.

Glucose



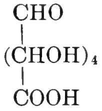
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{(CHOH)_4} \\ | \\ \mathrm{CH\ OH} \end{array}$$

Gluconic acid



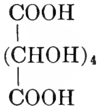
$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{(CHOH)_4} \\ | \\ \mathrm{CH\ OH} \end{array}$$

Glucuronic acid



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{(CHOH)_4} \\ | \\ \mathrm{COOH} \end{array}$$

Saccharic acid



$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{(CHOH)_4} \\ | \\ \mathrm{COOH} \end{array}$$

Of these three, glucuronic is the most interesting physiologically because it is often found in the urine of animals as a result of only partial oxidation of glucose. Ordinarily glucose is oxidized down to its final products of carbon dioxide and water, but when the animal is given substances like camphor and turpentine, which are difficult to oxidize, they are joined with glucose to form a glucoside, which is then oxidized to glucuronic acid and eliminated in the urine. Similar processes have been found in plants, and the acids which have been derived from such oxidations have been grouped together as "uronic acids" by recent investigators.

Among the substances which yield these uronic acids are gums and pentosans.

Linkage.—The sugars are easily connected to each other, with the elimination of water, to form more complex compounds. It is thus that the monosaccharides are connected to form a disaccharide or a trisaccharide:

$$2C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O$$

and these in turn to form the polysaccharides. When hydrolyzed the reverse process takes place:

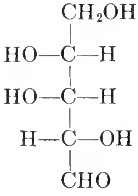
$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6.$$

Monosaccharides.—The monosaccharides are sugars which cannot be split or hydrolyzed into simpler compounds with the properties of sugar, that is, they are the simplest sugars. One group of monosaccharides, the pentoses, contains five carbon atoms while the other group, the hexoses, has six carbon atoms. In the laboratory, monosaccharides have been made with three and four atoms of carbon, but these do not occur free in plants.

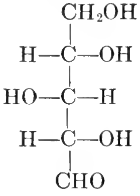
Pentoses.—The pentoses have the formula $C_5H_{10}O_5$. They have been reported in very small amounts in the free state in corn, turnips, bamboo, and other plants, but they generally exist in the combined state as condensation products from which water has been eliminated. These products, which are commonly gums and mucilages, yield pentoses when hydrolyzed, in much the same way that starch yields glucose. The two pentoses commonly found in plants are arabinose and xylose; they are not so readily fermentable by yeast as the hexoses.

Arabinose is found in cherry gum, peach gum, and gum arabic. It has a very sweet taste and is dextrorotary. *Xylose* is obtained by hydrolysis from wood gum and straw as well as from grains such as barley and corn. It is not so sweet as arabinose and is less dextrorotary. The formulæ for these pentoses are:

Arabinose



Xylose



Rhamnose ($CH_3C_5H_9O_5$) is a pentose in which one of the hydrogen atoms has been replaced by a methyl group.

Hexoses.—The hexoses contain six carbon atoms and have the formula $C_6H_{12}O_6$. Unlike the pentoses, all except galactose can readily be fermented by the enzymes of yeast, which break them down to alcohol and carbon dioxide according to the equation

$$C_6H_{12}O_6 = 2C_2H_5OH + CO_2$$

(Chap. XVI and XXV). Glucose and fructose are the only hexoses found as such in plants, but mannose, sorbose, and galactose occur in various complexes.

Since the common form of *glucose* is dextrorotary, it is also called *dextrose.* Grape sugar is still another name so that these three different terms are names for the same sugar. It is found in juices of ripening fruits and in honey, and is widely distributed throughout plants in roots, stems, and leaves. It is also produced by the hydrolysis of cane sugar, maltose, lactose, and cellulose, as well as from many other di- and poly-saccharides, all of which yield some glucose.

Dextrose is a white crystalline substance readily soluble in water and is doubtless one of the first products of photosynthesis, as discussed in Chapter VI. It is produced for the market from hydrolysis of cornstarch, and is the chief constituent of "corn syrup." In the U. S. A., 100–200,000 tons of dextrose are manufactured annually from corn. It is only about 60% as sweet as cane sugar, which, although soluble, must be broken down in the body to glucose before it can be utilized. For this reason patients with weak digestive powers are given glucose instead of sucrose, and in some sanitaria it is the form of sugar ordinarily served. It is also possible to buy candy and other sweets which contain glucose instead of sucrose.

Fructose, the other hexose found as such in plants, is also widely distributed in honey, fruits, and other plant organs. It rotates the beam of polarized light to the left and is hence called *levulose.* Another common name is fruit sugar. It is generally found along with dextrose, and both result in equal amounts from the hydrolysis of sucrose. Levulose turns the beam of light to the left (93° in a 10% solution at 20° C.) more than dextrose turns it to the right (52.5°). Since the original sucrose is dextrorotary (66.5°) and the mixture of glucose and fructose which results is levorotary, the mixture is commonly called invert sugar. Unlike glucose, levulose is soluble in alcohol and is a ketone instead of an aldehyde. About a fourth sweeter than cane sugar, it is the sweetest of the ordinary sugars. Jerusalem artichokes yield on hydrolysis 10–15% of levulose and now form the chief commercial source of this sugar.

Mannose does not occur free in plants, but is obtained from the hydrolysis of its condensation products, known as mannans or mannosans. It is obtained from the sap of the manna ash (*Fraxinus ornus*) and from the hemicelluloses of peas, coffee beans, date stones, and vegetable ivory nuts (*Phytelephas*).

Galactose occurs rarely, if ever, free in plants, but is a constituent of the trisaccharide, raffinose, and of the glucoside, digitalin. It is also commonly found associated with the pentoses in gums and mucilages. Agar-agar, which is a mucilage obtained from the red algæ, yields a high percentage of galactose, and the pectins of carrots and pears are also rich in this sugar. In the animal kingdom it occurs as one of the constituents of milk sugar, which consequently on hydrolysis yields galactose. It is only slightly sweet, but otherwise it resembles glucose in many of its properties.

Sorbose comes from the juice of the fruit of the European mountain ash (*Sorbus aucuparia*). It probably does not exist as such in the plant but is rather an oxidation product. It is not fermentable by yeast, but in other respects it much resembles fructose.

Disaccharides.—The disaccharides are formed by the union of two monosaccharides with the elimination of water:

$$2C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O.$$

They might be formed in one of three ways: (1) by linking the two alcohol ends of the molecules together, (2) by linking the two aldehyde ends or the aldehyde and ketone, or (3) by linking the aldehyde (ketone) of one with the alcohol of the other. If an aldehyde group is still free, as would occur in the first and third cases, the sugar is a reducing sugar; otherwise it is not. When acted upon by dilute acids, specific enzymes, or other hydrolyzing agents, the disaccharides are broken down into their component monosaccharides.

Reducing Sugars.—*Maltose*, also known as malt sugar, is found widely distributed in plant tissues although in small quantities. It is formed from the hydrolysis of starch, and is obtained in large amounts during the germination of barley and other grains. The action may be represented as follows:

$$(C_6H_{10}O_5)n + H_2O = C_{12}H_{22}O_{11} + (C_6H_{10}O_5)n_{-2}.$$

Maltose is white, soluble in water and alcohol, dextrorotary, and crystallizes in the form of slender needles. When hydrolyzed it yields two molecules of glucose.

Isomaltose is thought to be an isomer of maltose, but its properties have not been well determined. *Cellobiose* is a disaccharide formed in the hydrolysis of cellulose. *Gentiobiose* results from the hydrolysis of the trisaccharide, gentianose, which is found in the yellow gentian (*G. lutea*). It is also the sugar found in the glucoside amygdalin (Chap. XV).

Lactose or milk sugar is found only in animals. The corresponding plant sugar is melibiose, which is produced by hydrolyzing raffinose. Both these sugars yield glucose and galactose when hydrolyzed.

Nonreducing Sugars.—*Sucrose*, which is also called cane sugar, beet sugar, and saccharose, is the most common of all the sugars. It is the ordinary sugar of commerce and is found especially in quantities which can be commercially exploited in maple sap, cane and sorghum juice, and the root of the sugar beet. Sugar beets contain 15–20% of sugar, and individual beets have been known to contain 25–30%. The only common sugar which is sweeter is fructose. Sucrose is formed from one molecule of glucose and one of levulose, and when hydrolyzed breaks down into these two sugars. As previously mentioned, it is dextrorotary, but the resulting mixture of levulose and dextrose is levorotary and is hence known as invert sugar. Sucrose is the sugar commonly found in nectaries, but the bees change it to dextrose and levulose, probably by the means of formic acid or secreted enzymes, with the result that honey contains monosaccharides but little disaccharide.

Trehalose, which is dextrorotary and nonreducing, seems to serve the same function in plants lacking chlorophyll, e. g., the fungi, that sucrose does in the autotrophic plants. It resembles sucrose in that it is not directly fermentable by yeast, but differs from it in that it yields only glucose when hydrolyzed.

Trisaccharides and Tetrasaccharides.—As the name indicates, the trisaccharides (formula $C_{18}H_{32}O_{16}$) are built up of three molecules of a monosaccharide with the elimination of water according to the equation:

$$3C_6H_{12}O_6 = C_{18}H_{32}O_{16} + 2H_2O.$$

When hydrolyzed they yield three molecules of a monosaccharide or one each of a monosaccharide and a disaccharide. About five are well established as present in plants.

The only reducing sugar of this group is *xanthorhamnose*. This peculiar sugar, which is obtained from members of the buckthorn family (Rhamnaceæ), is composed of two molecules of the pentose, rhamnose, combined with one of glucose.

Raffinose is obtained from cotton seeds, barley, eucalyptus, and the sugar beet, which forms the chief commercial source. It is associated with sucrose and resembles it in many properties. When completely hydrolyzed it yields a molecule each of levulose, dextrose, and galactose.

Gentianose, from the yellow gentian, yields fructose and gentiobiose. *Melezitose*, an isomer of gentianose, is a very sweet sugar which comes from the sap of several conifers, including the European larch (*Larix europæa*) and Douglas fir (*Pseudotsuga*). It collects on the twigs in small white flakes, and when hydrolyzed it yields glucose and levulose.

Stachyose, which comes from the tubers of a mint (*Stachys tuberifera*), is a faintly sweet sugar which yields a molecule of levulose, one of dextrose, and two of galactose, and is thus seen to be a tetrasaccharide.

Polysaccharides.—The polysaccharides are composed of many molecules of a monosaccharide linked together with the elimination of water. The general formula may be expressed as $(C_6H_{10}O_5)_n$ or more correctly $(C_6)_n(H_2O)_{5n+1}$ and $(C_5)_n(H_2O)_{4n+1}$, depending upon whether the substance is derived from hexoses or pentoses. The value of n is probably between 30 and 200 but, owing to the slight solubility of these materials, their actual molecular weight is very hard to determine.

Hexosans.—The hexosans have the general formula given above, and are built up from the hexose sugars, which they yield on hydrolysis. There are four different groups depending upon the hexose which composes them; and upon this basis the hexosans may be classified as dextrosans, levulosans, mannosans, and galactosans.

The *dextrosans* or *starches* are the most common polysaccharides found in plants. The sugar produced in the green leaves as the result of photosynthesis is carried to all parts of the plant, where it is stored up in the form of starch as a temporary or permanent reserve material. Fleshy roots, the rays of the stem, and seeds are especially rich in this substance. In the cereals about 60% of the dry weight is starch, and in potatoes about 80%. The latex of laticiferous plants also contains much starch. However some plants among the monocots (*Scilla nutans*, *Phleum pratense*, *Allium*) contain very little starch, the reserves being stored either as fats or as sugars.

The starch occurs in grains of characteristic size and shape depending upon the species of the plant; and the starch granule is therefore one of the common criteria for detecting adulterations. The starch is often deposited in layers, the cause of which has not been definitely determined. Meyer proposed that the layers were due to differences in chemical composition, while Kramer has stated that they are alternations of colloidal and crystalline starch. Others have associated the layers with differences in water content and the alternation of day and night.

Starch is insoluble in water but when boiled yields a colloidal solution. Under the action of enzymes such as diastase, the starch is broken down through a series of simpler compounds until ultimately glucose is produced (Chap. XXII). The most characteristic reaction is the blue color which results when treated with iodine.

Dextrins may occur in plants as a form of stored food (*Arum italicum* and sweet corn, according to Weatherwax), but in general the dextrins are found as transition products between starch and maltose. Three such intermediate steps have been distinguished, known as amylo-, erythro-, and achroö-dextrin. Amylodextrin is the principal constituent of "soluble starch." It is readily soluble in hot water, has a starchy taste, and turns blue with iodine. Erythrodextrin is easily soluble in cold water, is tasteless, and gives a reddish-brown color with iodine. Achroödextrin is soluble in water, has a sweetish taste, and gives no color with iodine.

Commercial dextrin is made by heating starch to about 250° C. or by hydrolysis with acids. It is a mixture of glucose and the two simplest dextrins. It is used in library paste and on postage stamps, where one can recognize the faintly sweetish taste of the achroödextrin and the glucose.

Glycogen or "animal starch" is the common form of starch stored in the animal body. The liver is a glycogen storehouse but, although so common in animals, glycogen is much rarer in plants, where it is found chiefly in the fungi. Evidently there is some connection between the heterotrophic form of nutrition and the kind of carbohydrate stored. Yeast cells are especially rich in glycogen, which may account for some of the good results obtained from yeast in the animal diet. Glycogen resembles amylodextrin or soluble starch in its properties and on hydrolysis yields glucose.

Lichenin from lichens, paradextrin from a mushroom, *Boletus edulis,* and para-isodextrin from another fungus, *Polyporus betulinus,* are dextrosans which much resemble starch but which are of small economic importance. When hydrolyzed, lichenin yields cellobiose just as cellulose does.

The *levulosans* are built up from levulose in the same way that the dextrosans are built up from dextrose. Of these, by far the most important is *inulin,* which is found in many plants as the regular storage product. It occurs especially in the Compositæ (artichoke, chicory, and dahlia) but has been reported from many other families. In monocots, it has been observed that those in moist places tend to form starch, while those in the drier places are more likely to form inulin. This levulosan is a white, tasteless powder soluble in water, from which it may be precipitated by alcohol in the form of spherocrystals. It is levorotary and is hydrolyzed in the plant by (the enzyme) inulase to form fructose.

Other levulosans of minor importance in plants are graminin from grasses, irisin from *Iris,* phlein from timothy, sinistrin from *Scilla,* and triticin from *Triticum repens,* which from their gummy nature and general behavior are thought to correspond to dextrins.

Mannosans are polysaccharides which contain mannose, but most of these also contain other sugars such as galactose and dextrose. They are found in many plants as reserve food materials, e. g., in the leaves and wood of many trees such as the chestnut, apple, mulberry, and conifers. The seeds of many palms including the vegetable ivory palm contain in the hard outer walls these mannosans in combination with cellulose, with the result that these substances are commonly classified with the reserve celluloses. Mannan is the most common of the mannosans, but secalan from rye is also one.

Galactosans are similar complexes which yield galactose along with some other hexose or pentose. They occur together with the mannosans in reserve cellulose and have been found in the testas of beans and peas as well as in the regular reserve regions of the coffee bean, date seed, etc. Among the galactosans are galactan and paragalactan, both of which are white amorphous solids slightly soluble in water and hydrolyzed with difficulty.

Pentosans.—These substances are found in the stems and seed coats of many plants, where they are considered to be of prime importance in conserving moisture and in aiding the plant to resist low temperatures. Thus Hooker and Rosa (1920) found a distinct correlation between hardiness and pentosan content in fruit trees and vegetables. Corn bran contains about 40% and wheat straw about 25% of pentosans. They are white solids which are difficult to hydrolyze and are only slightly soluble in water. When finally broken down they yield the pentoses, arabinose and xylose, and thus bear the same relation to the pentoses that the hexosans do to hexoses.

Araban occurs in various gums such as gum arabic and cherry gum in combination with other materials. *Xylan* is the chief pentosan in lignified cell walls and is found in straw, corncobs, nutshells, seed testas, and similar regions, probably occurring wherever lignified tissue is found.

The *gums* are amorphous translucent substances, which are widely distributed in plants, especially in trees and shrubs of warm regions. They are composed of sugars (chiefly pentoses) united with an organic acid, which permits them to form salts with metals. Some are soluble in water, while others simply swell up forming a jellylike mass. Because they are so hard to hydrolyze, they are practically indigestible and are of little food value for animals.

Gum arabic is used commercially in the preparation of mucilage and as a base in many toilet articles. It is a brittle, amorphous, glassy exudate from the branches of Sudan acacias (*A. senegal* and *A. virek*) although other acacias yield inferior gums. When hydrolyzed it yields arabic acid, galactose, and arabinose. The formula, $C_{91}H_{150}O_{78}$, gives an idea of its complexity.

Gum tragacanth comes from another member of the legume family (*Astragalus*) and is used for very much the same purposes as gum arabic. When hydrolyzed it yields arabinose, xylose, galactose, and geddic acid, which is an isomer of arabic. It is obtained by wounding the stem, under which conditions the gum results from the metamorphosis of the medullary rays.

Cherry gum or *cerasin* is often found on the stems of the cherry, plum, and peach (*Prunus*). When hydrolyzed it yields almost entirely arabinose. Closely related to it are the wound gums which seem to be secreted by living cells of the wood in proximity to a cut or wound. Their function is not well known. In composition they are related to both gums and cellulose.

Mucilages.—The mucilages differ from the gums in that they do not dissolve in water, and from the pectins in that they do not gelatinize. They swell up in water and produce a slimy colloidal solution. They occur in many places throughout the plant kingdom:—in the roots and flowers of mallows (from which marshmallow comes); in onion bulbs; in the succulent leaves and stems of the *Agave, Yucca,* and cacti; in the fruit of the banana; in the seed of the mistletoe; in mucilage-secreting hairs; and in the walls of many of the algæ like *Spirogyra, Nostoc,* and *Laminaria.*

The chemical composition of the mucilages is not exactly known, but when hydrolyzed they yield pentoses and hexoses, chief of which are xylose, glucose, and galactose. Wiesner considers them to be formed from the action of an enzyme on cellulose.

Although classified as structural materials, the mucilages seem to have various uses in the plant. In aquatic plants they may aid to prevent too rapid outward diffusion of substances in the cell. When found on the epidermis they probably check transpiration, and in desert plants Spoehr and others have shown that they play an important rôle in retaining water within the plant, where, due to their water-holding capacity, they serve as an integral part of the storage mechanism. When found in tubers, as in the orchids, or in fruits, it is assumed that they act as reserve foods, although whether such difficultly hydrolyzable compounds can be used for food is doubtful. In connection with seeds such as *Salvia* and mistletoe, the mucilage aids in seed dispersal. The mucilaginous seeds of the mistletoe stick to the beaks of birds which have eaten the fruit and, when wiped off, remain upon the limb of the tree.

Here they are in the proper position for germination, the mucilage also holding sufficient water to start development.

Pectins.—In the cell wall of succulent acid fruits such as tomatoes, currants, gooseberries, strawberries, apples, peaches, and cherries (as well as in the petioles of rhubarb and the fleshy roots of carrots, beets, and turnips) is a carbohydrate called *pectose, protopectin,* or *pectinogen,* which, according to Fremy (1840), causes the hardness of the unripe fruit. As maturation proceeds, this pectose undergoes a series of hydrolytic changes, which ultimately result in the formation of pectic acid. Among the principal intermediate products are the *pectins,* which (like pectic acid) are insoluble in water, especially if acids are present, and readily form gels in the presence of calcium salts. The jelling of fruit juices is due to the presence of these pectins and pectic acid. Also in fruits which discharge the seeds to some distance when ripe, e. g., *Oxalis, Impatiens, Dorstenia,* and *Lathræa,* the fruit walls have been found to be rich in pectin (Overbeck, 1924), the swelling of which probably aids in this dehiscence.

The composition of pectins is not well known. Among the sugars finally produced by hydrolysis are arabinose and galactose, while the presence of a "uronic" acid shows the relation of the pectins to the gums. The pectoses have been of prime interest to the plant pathologist, since many of the worst fungal parasites gain entrance to the cell by dissolving the middle lamella, which is made of either pectose or pectocellulose. This pectose (which is probably either a calcium salt of pectic acid, an insoluble ester of that acid, or a compound of a pentosan and a pectin) may serve as food for the fungus; but more frequently the food is derived from the contents of the cells themselves.

The enzymes which play a part in these various processes are somewhat confused in the literature. The enzyme which dissolves the middle lamella and breaks it down to pectins is called *pectosinase* or *protopectinase. Pectase* is the enzyme which hydrolyzes pectins to pectic acid and, in the presence of the calcium salts, brings about the formation of the gel (calcium pectate). *Pectinase* hydrolyzes pectin (and presumably also pectic acid) to the simple components—the sugars and galacturonic acid—mentioned above.

Celluloses.—The term cellulose is used in two ways. First, it means a specific chemical compound with distinct properties. Secondly, it is employed in a general sense to include not only the cellulose itself, which makes up the cell walls of most plants, but the other substances which, in the course of the maturation of the cell wall, become associated with it such as lignin, cutin, suberin, etc. With this in mind, cellulose may be classified as follows:

1. Normal cellulose.
2. Compound cellulose.
    a. ligno.
    b. pecto.
    c. cuto.
3. Reserve cellulose (also called *hemi* and *pseudo*).

Normal or true cellulose is exemplified by that found in cotton fiber, which is 99% pure cellulose, as well as that found in flax, hemp, etc. The cotton fibers are freed from the small amount of impurities by treating with alkali, leaving pure cellulose ($C_6H_{10}O_5n$), which is a white hygroscopic substance insoluble in water, typified by good paper, cotton wool, etc. When hydrolyzed by the enzyme *cytase* it is broken down into cellobiose and then into glucose. The action of certain bacteria on the cellulose teeth of the leaves of *Elodea,* one of the water plants, can be easily seen with the microscope; and it is a matter of common observation that plant stems left in the ground decay in the course of time producing in the soil the so-called "humins." Under stagnant water as in swamps and similar places, the cellulose is broken down into carbon dioxide and marsh gas according to the equation:

$$C_6H_{10}O_5+H_2O=3CO_2+3CH_4.$$

In the digestive tract of animals such fermentations sometimes occur, producing in addition hydrogen, under which circumstances the animal becomes "bloated"; and in 1923, Khouvine even isolated cellulose bacteria from the human intestine.

Lignocellulose is formed after the cells of the wood have reached their ultimate length, and when the cellulose of the wall becomes impregnated with a substance known as *lignin,* which gives to wood its peculiar nature. The cellulose, in other words, has become lignified. Cheap qualities of paper such as that in newspapers are made from lignocellulose. They give the lignin tests and turn yellow in the light.

Pectocelluloses are the compounds of pectic acid and cellulose previously mentioned as occurring in the cell walls of many stems, storage roots, fruits, and seeds. They are about the same as the pectoses. By some workers lichenin is considered to be a pectocellulose and is classified here rather than with the dextrosans (page 138).

The *cuto-celluloses,* which occur in the walls of cuticularized cells thus rendering them waterproof, have been supposed to be cellulose impregnated with cutin, which, along with waxy substances, forms the cuticle. Investigations of Gilson (1890), Van Wisselingh (1893), and more recent workers (Sando, unpublished work) tend to show, however, that no cellulose is chemically combined with the cutin, in which case these compounds should be dropped from the category of celluloses.

The *hemi-* or *pseudo-celluloses* are compounds which occur in the seed coats of peas and beans, in coconut shells, date seeds, etc. They are much more easily hydrolyzed than other celluloses and give rise to mannose, galactose, and pentoses. They bear the same relation to these sugars that starch does to glucose and, for this reason, are practically the same as the mannosans, galactosans, and pentosans already considered. They seem to function as a kind of reserve material stored in the external structures of the testa and shell rather than in the internal parts of the seed such as the endosperm and embryo. Also, according to Murneek (1929), they may act as important reserves in woody plants, notably in fruit trees.

Conclusion.—The foregoing pages have been an attempt to give a brief summary of the carbohydrates found in plants, their chemical relationships, and their place in the physiology of the plant. They are the first products formed and from the point of view of energy changes the most important.

Why some plants should store their food in one way and some in another is not entirely clear, although, in some instances, it is easy to see a survival value in the method adopted. Cold weather means physiological dryness for the plant, owing to the fact that water in the form of ice is unavailable, and favors the storage in a soluble form such as sugar rather than an insoluble form like starch. In aquatic plants which hibernate at the bottom and are killed by freezing weather, e. g., *Elodea,* the food is in the form of starch; while those like *Myosotis,* which live through the winter, even when the temperature reaches −7° C., contain sugar during the cold season even though they may have starch in the summer. Arctic plants contain relatively large quantities of sugar and less of starch. In the ordinary Irish potato, when the tubers are exposed to temperatures as low as 7° C., the starch is transformed into sugar. As much as 3% of sugar may be formed, but when the temperature is raised the sugar which is not respired is turned back into starch. In maple trees the food is stored in the form of sugar, which is rapidly converted into starch in the spring upon the approach of warm weather. Lidforss, by feeding sugars to hothouse plants, succeeded in making them resistant to −7° C. in some cases.

While the presence of sugar seems to be associated with low temperatures and assists the plant to keep a high osmotic pressure so that the proteins are not precipitated by freezing (Chap. XIX), some plants, like the sugar beet, are rich in sugar and nevertheless rather sensitive to cold weather. However, cool weather facilitates the transfer of sugar to the root, which explains in part the favorable effect of cool autumns upon the percentage of sugar in beet roots. Although the sugar beet is not very hardy, it is doubtless hardier than it would be without the sugar.

QUESTIONS

1. What are the two large classes of carbon compounds? How do they differ?
2. Discuss the construction of the polarimeter.
3. What is reduction?
4. What is the characteristic grouping (radical) in an organic acid?
5. How many kinds of isomers are found in the sugars?
6. Why is a knowledge of the chemistry of the carbohydrates necessary for a student of plant physiology?
7. Why is the maple sugar season so short?
8. Why do potatoes sometimes get sweet in cold weather?
9. How do pentosans help to make the plant resistant to low temperature? to dry weather?
10. Are all sugars equally usable by the plant? (See Knudson, 1926.)

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CHAPTER XIII

FATS, WAXES, AND LIPOIDS

> Where it is a duty to worship the sun, it is pretty sure to be a crime to examine the laws of heat.
>
> —VOLTAIRE.

As mentioned in Chapter III, there are other ternary foods (foods composed of three elements) besides the carbohydrates, viz., the fatty substances. Although they contain the same elements as the carbohydrates, they have relatively less oxygen and more hydrogen. The fatty foods are commonly divided into fats and oils, but there is no chemical difference between a fat and an oil. The term "oil" is simply used to designate those fats which are liquid at ordinary temperatures and thus it denotes a physical state rather than a chemical combination. The mineral oils such as kerosene, gasoline, etc., are not related and belong to an entirely different category of substances.

Economic Uses of Fats.—Fats are common in storage organs throughout the plant kingdom, especially in seeds. The following table of plants with the percentage of contained fats will give an idea of the wide range of families where fats are found in large quantities.

Almonds 42%
Brazil nuts 68%
Castor beans 51%
Cocoa 54%
Coconut 65%
Coffee 12%
Corn 4%
Cottonseed 24%
Flaxseed 30%
Hazel nuts 55%
Hempseed 33%
Mustard seed 25%
Olives 50%
Peach pits 35%
Poppy seed 47%
Pumpkin seed 41%
Rapeseed 42%
Sunflower seed 38%
Walnuts 52%.

Among these are many fats and oils of commercial importance, which are used for food, lighting, lubricating, manufacture of soaps, and many other purposes.

Castor oil is used in the dye industry after conversion into "turkey red" oil. It is also used in medicine as an internal lubricant and since the development of aviation has been much in demand as a lubricant for air motors.

Coconut oil, from the seeds of the coconut, is commonly called copra oil. Copra soaps can absorb large quantities of salt solution and are hence in demand for washing with sea water. Palm oil, which comes from one of the African palms, has the consistency of lard and turns yellow when exposed to the air. It is used in soaps and also has been used in margarines for food, but this is now illegal in the United States.

Cottonseed oil, which is extracted from the seed of cotton, is used in foods as a substitute for olive oils or when hydrogenated for lard. It is also used in the manufacture of soap and certain rubber substitutes.

Linseed oil, from the seed of flax, is used chiefly in the manufacture of paints, varnishes, and printer's ink because of its rapid drying qualities. Other oils, such as walnut and poppy oil, are used especially for the paints of artists.

Olive oil, which is extracted from the *fruit* of the olive, is used largely in southern Europe for cooking. Poorer grades of oil are used in the soap industry.

General Characters.—The fats are all lighter than water and are insoluble in it. They dissolve readily, however, in ether, chloroform, and carbon disulphide. They may be saponified, as described below, and they leave a permanent translucent stain on paper.

They are all esters of the trihydric alcohol, glycerol. In the chapter on the chemistry of chlorophyll it was pointed out that an ester is a "salt" of an alcohol and an acid. Just as sodium chloride is formed by the union of sodium hydroxide (NaOH) and hydrochloric acid (HCl) with the release of water:

$$NaOH + HCl = NaCl + H_2O$$

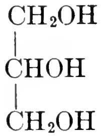
so, e. g., ethyl alcohol may combine with formic acid to form the ester, ethyl formate, and water:

$$C_2H_5OH + HCOOH = C_2H_5COOH + H_2O.$$

This general reaction between an acid and an alcohol may be represented thus:

$$R.OH + H.OOCR = R.OOCR + H_2O.$$

The alcohol found in fats is the trihydric alcohol called *glycerol,* which is obtained on the market as *glycerin.* Its formula is

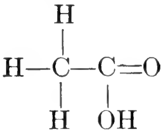


$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

Glycerol is a very heavy, colorless, viscid, sweetish liquid, which is freely miscible with water in all proportions. It is used extensively in the manufacture of explosives such as dynamite and nitroglycerin, and is obtained from fats by a process known as *saponification,* which consists in treating the fat with an alkali. As a result the glycerol is separated out, and the alkali unites with the fatty acid forming a soap. Whether or not the soap formed can be used as such, the process is called saponification and the salt of the alkali and acid is a *chemical* soap.

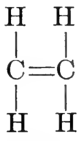
Fatty Acids.—Among the common fatty acids found in various fats are formic ($H.COOH$), which is found in nettles and also in the bodies of ants and bees; acetic ($CH_3.COOH$), which occurs in vinegar; butyric ($C_3H_7.COOH$), which is the common acid of butter fat and occurs free in rancid butter, when the fat has been broken down into its component parts; palmitic ($C_{15}H_{31}.COOH$), which is common in palm oil and many fats; and stearic acid ($C_{17}H_{35}.COOH$), which is present in most fats and oils.

It will be observed that the constitution of the above fatty acids can all be expressed by the general formula $CnH_{2n+1}.COOH$. In all the fats found in nature none have been obtained where n was other than an *odd* number which makes the total number of carbon atoms in the molecule *even.* Further, if we examine the structure of one of the above acids, e. g., acetic acid



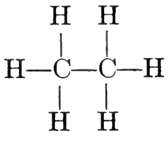
$$\begin{array}{ccccc} & & H & & \\ & & | & & \\ H & — & C & — & C=O \\ & & | & & | \\ & & H & & OH \end{array}$$

it is observed that the carbon atoms are all *saturated,* by which is meant that each of the four valencies of the carbon atoms are completely "satisfied." The difference between "satisfied" and "unsatisfied" valencies may be illustrated by the formulæ of ethane and ethylene:



$$\begin{array}{ccc} H & & H \\ | & & | \\ C & = & C \\ | & & | \\ H & & H \end{array}$$

ethylene



$$\begin{array}{ccccccc} & & H & & H & & \\ & & | & & | & & \\ H & - & C & - & C & - & H \\ & & | & & | & & \\ & & H & & H & & \end{array}$$

ethane

In the ethylene it is seen that there are not enough hydrogen atoms to satisfy all the valencies, and in order that none may be left unsatisfied the remaining valencies must be joined to each other, thus uniting two carbon atoms by more than a single bond. Such compounds are said to be *unsaturated*, that is, they can easily take on more atoms before their full quota would be used up, according to the valencies of the atoms present.

The above fatty acids are all saturated and belong to the acetic or stearic acid series. Other series, such as the oleic and linoleic series, are unsaturated. In the oleic acid series are oleic acid ($C_{17}H_{33}.COOH$) from olive oil, and brassic acid ($C_{21}H_{41}.COOH$), which occurs in rape and mustard oil. The general formula is $CnH_{2n-1}.COOH$, and they are thus seen to be unsaturated.

The linoleic acid series has the general formula $CnH_{2n-3}.COOH$ and is represented by linoleic acid ($C_{17}H_{31}.COOH$) found in linseed oil. The explanation of the drying properties of this oil is now obvious. Since it is so far from being saturated, it takes on oxygen readily when exposed to the air and increases in weight, at the same time becoming stiff and hard. For this reason those fats containing a high percentage of unsaturated acids are called "drying" oils and are used largely in paints, varnishes, etc., while those which are more nearly saturated are nondrying and are used more for food and lubricating purposes.

As previously mentioned, the fatty acids all contain an even number of carbon atoms. About 80% of the acids of the glycerides which make up the fats of plants and animals are those such as oleic, linoleic, ricinoleic, and stearic acids, all of which contain 18 carbon atoms. This is a multiple of six and must bear some relation to the fact that the principal starches and sugars also contain multiples of six carbon atoms in their molecules. In any discussion of the origin of the fats and their relation to the carbohydrates, this should be borne in mind.

As will be seen from the formula of glycerol, there are three hydroxyl (OH) groups which can unite with the fatty acid. All of these may unite with the same acid to produce a simple fat (yielding only one kind of acid) or each may be united with a different fatty acid so that when broken down two or three different fatty acids will result (oleostearin, stearopalmitin, etc.). In this way it is seen that the number of fats produced with the various kinds of fatty acids in different combinations is enormous. Furthermore, only one or two of the hydroxyl groups of the glycerol may combine with a fatty acid.

It is interesting to note here that the fats with saturated fatty acids have a higher melting point than those with unsaturated acids. This means that those of the latter group will be liquid at lower temperatures than those of the former. It is, therefore, not surprising to find that the plants of the tropics have fats rich in saturated acids, while those of the temperate and colder regions of the earth contain more of the unsaturated acid groups. Thus in cocoa butter, the chief fatty acid is stearic, while in olive oil it is oleic. The oils of the pines are liquid at such low temperatures as −30° C., but in the peanut, the fat is in the solid condition below 2° C. Since materials in a solid state are difficult to transport and to utilize in the plant, the importance of the type of fat from a metabolic point of view is evident.

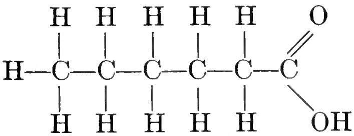
Origin of the Fats.—The origin of the fats in the plant has been the subject of much research the past few years and concerning it there are many hypotheses but little definitely established material. It is now quite generally conceded that the fats are built up from the fatty acids and glycerol by means of the enzyme, *lipase.* In the laboratory this synthesis has been accomplished by heating for some time under pressure at 220° C. The problem then becomes one of determining how the glycerol and fatty acids are made.

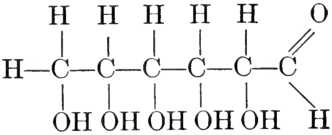
Although Fleissig thinks that in *Vaucheria,* which contains much oil, the oil arises as a direct photosynthetic product much as sugars are formed in ordinary plants, the majority of workers agree that the fats are secondary products formed from the carbohydrates. Since carbohydrates are the first materials made by the plant, it seems reasonable to suppose that the fats come from them, and experimental evidence for this view is not lacking. In animals it is a common practice to "fatten" stock by feeding carbohydrates. Sugars are commonly spoken of as being "fattening" and during the sugar season in cane-growing regions, the Negroes who work in the cane fields get noticeably fatter. All this tends to show a connection between the fats and the carbohydrates, but more definite experimental evidence is at hand. Schmidt (1891), Le Clerc du Sablon (1893–1897), Ivanov (1911–1912), and others have shown with castor beans, peony, almond, walnut, rape, and other fatty seeds, that the fat makes its appearance in the storage organ as the plant approaches maturity, and that this appearance of the fat is accompanied by a decrease in the carbohydrates present. For the almond the following figures (from Le Clerc du Sablon) are given, which are typical of other plants as well:

Date of gathering	*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
Aug. 1	37	0	2.8	6.2
Sept. 1	44	0	2.6	5.4
Oct. 4	46	0	2.5	5.3

Glucose, sucrose, and starch appear to be the foods commonly used for this transformation, the latter being first hydrolyzed to monosaccharides. In many evergreens similar changes from carbohydrates to fats are observed during cold weather, the carbohydrates reappearing on the approach of warm weather.

How are the carbohydrates transformed into the fatty acid and the glycerol? If we compare the formulæ of a hexose and glycerol, it is easy to see how two molecules of glycerol could be derived from one of glucose. During the World War methods of producing glycerol from sugar by means of fermentation were developed to a high degree of perfection by the Central Powers. The more difficult problem is to form the organic acids from sugar. If a hexose is compared with a fatty acid containing six carbon atoms, such as caproic acid, it would seem not impossible to produce one from the other. Two changes only would be needed.

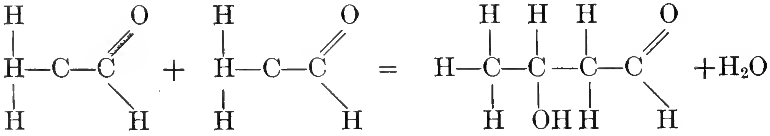

caproic acid


a hexose

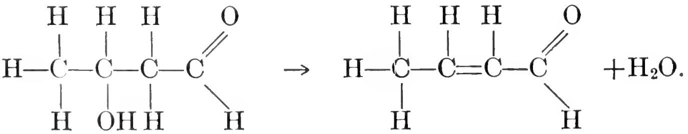
First, the hydroxyl groups must be reduced to hydrogen and, secondly, the terminal aldehyde must be changed into an acid group. Simple as it looks, it is very hard to do, and how the plant does it we have no exact idea. It is possible that hexoses are transformed into six carbon acids and these acids then linked together, or the acids might possibly be made directly from 18 carbon sugars (trisaccharides). The significance of the fact that the common sugars and the common acids contain multiples of six carbon atoms has been mentioned above.

Smedley (1912) has prepared fatty acids from carbohydrates by first breaking the hexoses into three-carbon compounds and then oxidizing these into pyruvic acid ($CH_3.CO.COOH$), which is then condensed into acids with longer carbon chains. The difficulty with which this is accomplished is in sharp contrast to the ease with which the plant accomplishes these transformations, and that the plant uses this method is doubtful, although Neuberg has isolated an enzyme which he calls carboligase that can unite two molecules of pyruvic acid or of acetaldehyde.

A somewhat similar theory (which, however, has met with more success) is that of Raper (1907) and Euler (1909), who proposed that the synthesis of fatty acids from sugar takes place by breaking the sugars down into acetaldehyde and then forming the acids from this substance. The fact that acetaldehyde has been found in the products of sugar decomposition in cells lends decided support to this hypothesis, according to which two molecules of acetaldehyde unite first to form an aldol:



which changes to crotonic aldehyde by removal of water



Another molecule of acetaldehyde is added to this with the subsequent removal of water, and the product sorbic aldehyde is formed, which has been found in plants. Three molecules of sorbic aldehyde can be linked together to give an unsaturated aldehyde that can be easily changed to the fatty acids.

Utilization of Fats in the Plant.—The problem has been attacked by noting the changes which take place in fatty seeds during germination, the assumption being that the reverse change takes place when the fat is built up. Miller (1910) showed that the resting sunflower seed contains less than 1% of fatty acid, but when germination has proceeded far enough for the plumule to show, the fatty acid reaches 30%. Glycerol does not show so readily because it is more easily translocated and consequently harder to detect.

Ivanov (1912) found that fatty acids increased considerably during germination and then disappeared, the unsaturated ones disappearing first. He believes this is due to the fact that the unsaturated ones are easily transformed into sugars before being oxidized, while the saturated ones are oxidized without being changed into sugar.

Le Clerc du Sablon found the resting seed of castor bean contained 69% of oil and 4% of sugar, while in a seedling 11 cm. high the oil had fallen to 11% and the sugar had risen to 14%. The kind of sugar formed depends upon the nature of the oil. Detmer has suggested that the kind of action which takes place might be represented by the following equation:

$$\underset{\text{oleic acid}}{C_{18}H_{34}O_2}+27O=2(C_6H_{10}O_5)+6CO_2+5H_2O$$

in which case a carbohydrate, carbon dioxide, and water are produced by the oxidation of the fatty acid. It is only reasonable to suppose that the reverse reactions may take place in the building up of fats.

Fats as a Source of Energy.—In animals, fats are the chief source of stored energy. In plants, however, they are not as important on the whole as carbohydrates, but are found especially in seeds, where much energy must be stored in a small space. Since the fats contain more hydrogen and less oxygen than the carbohydrates, they are able to take on more oxygen and release more energy in the oxidizing process than the carbohydrates. One g. of fat when oxidized gives an average of 9.3 calories of heat energy, while one g. of starch gives only 4.1 calories. Thus, while enough fat to yield 100 kg. cal. of heat can be placed in 12 c. c., at least twice that much space would be needed to contain starch for the same amount of energy when oxidized. This gives an idea of the advantage of storing energy in the form of fats when there is not much available room, and it is, therefore, not surprising to learn that more than 75% of all plants store reserves in the seed in the form of fats or oils, where they compose at least a third of the dry weight. As stated above, just how much of the fat is oxidized as fat and how much is oxidized as carbohydrate, after first being transformed into the sugars, is a disputed question. According to Maquenne and Ivanov, the saturated fatty acids are oxidized directly, while the unsaturated ones are first changed to sugars.

Appearance of Fats.—The fats first appear in the protoplasm as small vacuoles, which later run together to form larger drops. In some cases the oil has been described as originating from the activity of small plastids called *elaioplasts*, which are supposed to bear the same relation to oil as leucoplasts in the potato bear to the formation of the starch grains. These plastids have been observed in *Vanilla*, *Ornithogalum*, *Gaillardia*, and other plants. Beer (1909) thought that the elaioplasts were formed from degenerate chloroplasts, but more recent workers have connected the elaioplasts with special chondriosomes, from which they originate in much the same manner as the chloroplasts.

The Waxes.—The waxes differ from fats in that they are esters of fatty acids with *monohydric* alcohols of high atomic weight, e. g., ceryl alcohol ($C_{26}H_{53}OH$), instead of with glycerol, a trihydric alcohol. In common use the word wax is used to mean a harder substance than a fat, but these physical differences are not the correct criteria of differentiation. Thus what is commonly called "wool-fat" is a wax, and "Japan-wax" is a fat. The waxes, in addition to being compounds of monohydric alcohols, differ from the fats in being less easily hydrolyzed and less soluble.

The better known animal waxes are beeswax, spermaceti, and wool wax. Carnauba wax from the leaves of the wax palm (*Copernicia*) is the best known of the vegetable waxes. Poppy wax is an ester of ceryl alcohol and palmitic acid.

Because of their imperviousness to water, waxes are commonly found on leaves and fruits where there is need to check excessive evaporation. They are also often found on fruits such as plums and cherries, where they form what is called the *bloom*. Their efficiency may be easily determined by comparing the water loss from a fruit from which this glaucous covering has been removed with that from one on which it has been left intact. They are also very resistant to the attacks of fungi; and strawberries picked in the morning when the wax is firm have been found to rot much less than those picked later in the day when the wax was softened.

Sterols.—The monohydric alcohols of high atomic weight mentioned in the preceding section are straight-chain alcohols. Those of high atomic weight that are cyclic (aromatic series) are commonly called *sterols.* While the sterols themselves are not fats, they are frequently associated with the fats and produce their predominant physiological properties. For these reasons they may be discussed at this point. Cholesterol ($C_{27}H_{45}OH$) is the most common sterol in animals but has not yet been found in plants. Among the plant sterols (phytosterols), sitosterol, in the embryo of the cereals; stigmasterol, from various plant oils; and ergosterol, first found in ergot and later throughout the plant kingdom, are best known. Ergosterol has been publicized much more than any of the others because it was found (1926) to be the precursor of vitamin D (Chap. XVII). When irradiated with ultra-violet light, vitamin D is formed from this sterol, which is considered by Windaus to be an isomer of the vitamin. The irradiation causes a rearrangement of the molecule and the resultant vitamin properties.

Lipoids.—The name lipoid has been given to substances of a fatlike nature found in small quantities widely distributed in plants and animals but in rather larger amounts in special places such as nerve and brain tissue, egg yolk, and the seeds of plants. The lipoids are extracted from tissues by the same solvents as fats and, when hydrolyzed, yield fatty acid and a nitrogen compound. One group, in addition, yields phosphoric acid. The name lipoid means "fatlike" and was given because of the above-mentioned similarities to the fats.

Lipins.—This group of lipoids includes those which yield fatty acid and a nitrogenous compound but no phosphoric acid. Those found in the brain have been called *cerebrosides* but others are also found in plants. Galactose has replaced a part of the glycerol as the alcohol part of the ester in all the cases thus far reported.

The Phosphatids.—The phospholipins or phosphatids are the more common lipoids. They yield phosphoric acid in addition to the fatty acid and nitrogen compound. Of these, the best known and most common is *lecithin,* which occurs in the cereals, leguminous seeds, the brain, the heart, and the liver of animals, and in egg yolk. In fact, it is thought that every living cell contains some lecithin, but it is especially abundant where fats occur. Lecithin is an ester of glycerol and fatty acids in which one of the fatty acids is replaced by phosphoric acid, which, in turn, has one of its hydrogen atoms replaced by the nitrogen base, choline. Its composition may be illustrated thus:

$$\text{glycerol}\left\{\begin{array}{l}\text{fatty acid.}\\ \text{fatty acid.}\\ \text{phosphoric acid}+\text{choline.}\end{array}\right.$$

The fatty acids are different in different lecithins. In some lupine seeds, the fatty acids are reported as palmitic and stearic and the composition of the lecithin would then be:

$$\text{glycerol}\left\{\begin{array}{l}\text{palmitic acid.}\\ \text{stearic acid.}\\ \text{phosphoric acid}+\text{choline.}\end{array}\right.$$

Other phosphatids have been found in plants besides lecithin. In many of these, various carbohydrates replace all or a part of the glycerol as the alcoholic part of the ester. Among the carbohydrates thus identified have been galactose, dextrose, and pectose, which may vary from mere traces to 15% of the weight of the material from which the phosphatid was prepared. Such phosphatids have been found in potatoes, whole wheat flour, and pollen. Other nitrogen compounds may also be found instead of the choline; and, in addition to phosphorus, other minerals such as calcium and iron may be present.

Physiological Significance.—The lipoids are so universally present in tissues and so commonly found where the vital processes are the most pronounced, e. g., in the brain, heart, egg yolk, and seeds, that they must play some part in the activity of living protoplasm. Experimental studies have been made for the most part on animal cells and as yet there is little definite knowledge concerning their physiological importance. The fact that the lipoids are hard to isolate and properly purify for experimental purposes makes the problem all the harder. To date, only hypotheses have been advanced, among the more important of which are the following:

1. Overton (1901) suggests that the surface layer of all cells contains lipoids which, owing to the fact that they lower the sur-

face tension, collect at the surface in a very thin film (Chap. II). Here they regulate the passage of salts and other materials into the cell, since only those materials can come in which are soluble in lipoid. Although this theory has been supported by Hansteen-Cranner (who laid especial emphasis upon the rôle of the phosphatids) and many other investigators, Osterhout and others have pointed out that this theory seems hardly in accord with the fact that the substances which the plant actually needs, such as the watery solution of salts and sugars, are not soluble in lipoid and would be kept out of the cell, while alcohol and many detrimental materials would enter. The lipoids would seem to determine *im*permeability rather than permeability, and this, in fact, is the case with seed coats, as reported by Denny (1917), who found that testas rich in lipoids were much less permeable to water before the lipoids were extracted than afterwards. Overton, who based much of his work upon experiments with dyes, found that those dyes which entered the cell were soluble in lipoids while those to which the cell was impermeable were not. But the dyes which Overton used to support his theory have also been found to differ in their electric charges, so that no valid conclusions can be drawn from these results (Chap. XI).

2. Clowes has suggested that the lipoid surface may be the continuous phase of the protoplasmic emulsion at the surface of the cell only a part of the time and thus has tried to eliminate some of the difficulties in Overton's original hypothesis.

3. It has been proposed by Green and Jackson (1906) that the lipoids play an important rôle in fat metabolism. As the seeds of the castor bean germinate, the oil diminishes while the fatty acid and lecithin increase, as shown by the following table:

Degree of development	*Per cent oil*	*Per cent fatty acid*	*Per cent lecithin*
Resting seeds	82.8	2.2	0.236
Testa just cracked	67.5	4.6	0.17
Radicle 1–2 cm. long	52.5	11.9	0.475
Root system established	23.6	16.89	0.873

If lipoids are formed from fats during germination they may play a part in their formation.

4. Bang (1911) has pointed out that the lipoids are among the most labile of all the components in protoplasm. The many reactions in the plant require more active materials than either the fats or proteins, which are relatively stable substances; and the

lipoids, which contain both nitrogen and fatty acids, perhaps play an important rôle in bringing about the rapid changes so characteristic of the cell.

5. It has been proposed that lipoids are an intermediate product between fats and proteins. The presence of the fatty acid from the fats, and of choline and other nitrogenous compounds from protein decomposition lends a possibility to this idea. Fats develop in cheese, but according to Nierenstein (1911) such fats come from cholesterol and not from the proteins.

6. The view has been advanced by Korsakova (1910) and others that lipoids are concerned with the formation of enzymes, especially the proteases, and initiate the changes which lead to the formation of the enzymes from the zymogens (enzyme formers).

7. Palladin (1910) believed that respiration was dependent upon lipoids. When wheat seedlings were treated with various lipoid solvents, the greater the amount of lipoid extracted the less carbon dioxide was formed.

These are some of the views concerning lipoids to be found in the literature, but their real function and importance is one of the matters which wait upon further research for their ultimate solution.

QUESTIONS

1. What is an ester? saponification?
2. Why are fats so important in the conduct of war?
3. What is the difference between a saturated and an unsaturated compound? Give illustrations not discussed in the text.
4. Some trees are called "fat" trees and some "starch" trees. Give examples and explain the difference.
5. Which is the more concentrated food,—fat or sugar? Explain.
6. Which is the more quickly available? Why?

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CHAPTER XIV

PROTEINS

All things change, nothing perishes.
—Ovid.

Composition.—The proteins are organic substances composed of carbon, hydrogen, oxygen, nitrogen, and sulphur. They are used chiefly in building up the living material, protoplasm, and their name (Gr., *fundamental*) indicates their importance as the basis of the protoplasm itself. The vital phenomena take place in the protoplasm, and seem to consist largely of the reactions between the various parts of this proteinaceous material. Naturally proteins must occur in every living cell. Reproductive cells are almost entirely protein. As storage products they are found especially in seeds, those of leguminous plants being exceptionally rich.

In ordinary cells, the protein may occur in solution but, in storage organs, it is commonly found in small solid masses called *protein* or *aleurone* grains, which may be amorphous, crystalline, or partly both. Grains of the latter type are found in the castor bean (*Ricinus*). In the center of the amorphous protein matrix is a crystal of protein, at the end of which is a spherical "globoid" composed of a double phosphate of calcium and magnesium. The crystals of different proteins may be cubical, hexagonal, or needle-shaped; while many aleurone grains are much simpler and contain neither a crystal nor a globoid.

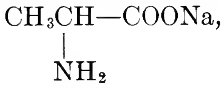
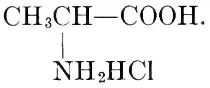
As mentioned above, the proteins are composed of five elements of which the sulphur occurs in the smallest proportions. Plant proteins are fairly uniform in their composition and the analysis of about twenty different ones gives the following average percentages of their constituents: carbon 52%, hydrogen 7%, nitrogen 17%, oxygen 22%, and sulphur 1%.

General Characters.—The general properties of the proteins may be catalogued and discussed under the following heads:

I. Chemical properties.
  a. amino-acid constitution.
  b. precipitation reactions.
  c. color reactions.

II. Physical properties.
  a. indiffusibility.
  b. coagulation.
  c. optical activity.
  d. precipitation without change.

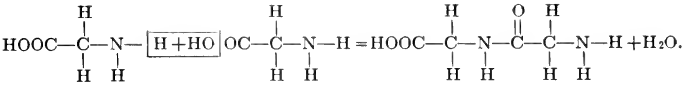
Amino-Acid Constitution.—The best definition which can be given of the simple proteins is that of Panzer (1903), who defined proteins as "all substances which upon hydrolysis yield simple amino acids." The amino acids are organic acids with an NH or an NH_2 group substituted for one of the hydrogen atoms. Thus if in acetic acid (CH_3COOH) we substitute an NH_2 group for one of the hydrogen atoms we get the amino acid, *glycine* (CH_2NH_2COOH). Similarly propionic acid (CH_3CH_2COOH) with hydrogens attached to two carbon atoms might give two amino acids,—CH_3CHNH_2COOH and $CH_2NH_2CH_2COOH$. The first, with the substituted NH_2 near the carboxyl end, is called *alpha*-propionic amino acid or *alanine*, and the other *beta*. While these two are possible, in natural proteins only the alpha amino acids are found, although there may in addition be substituted NH_2 groups on other carbon atoms as well, e. g., *lysine* ($CH_2NH_2CH_2CH_2CH_2CHNH_2COOH$). It is also possible to substitute NH_2 groups for more than one hydrogen on a single carbon atom, but such products are not found among the amino acids derived from natural proteins.

Owing to the presence of the amino acids, the proteins are given peculiar properties. If an amino acid is examined closely it will be seen to possess both basic and acid qualities. The carboxyl end is acid and can unite with bases forming salts of the base. The NH_2, however, is basic and can combine with acids to form salts of the acid. The amino acid can thus form salts with either an acid or a base. The amino acid, alanine, can consequently combine with sodium to form sodium-amino-propionate,  $CH_3\underset{\substack{|\\ NH_2}}{CH}—COONa$, and can also unite with hydrochloric acid to form alanine hydrochloride,  $CH_3\underset{\substack{|\\ NH_2HCl}}{CH}—COOH$. In the one case the amino acid has behaved like an acid and in the other like a base. Such substances are called *amphoteric*, and the proteins which result from the amino acids have this same property.

Because of this dual nature of the amino acids, two of them can be linked together with the elimination of water according to the equation:

M. Molliard, Professor of Plant Physiology, University of Paris, France. *Physiology of the fungi. Professor Molliard is the author of the best work in French on plant physiology.*

Dr. H. S. Reed, Professor of Plant Physiology, Citrus Experiment Station, Riverside, California. *Plant nutrition; Nature of growth.*



$$\mathrm{HOOC{-}\overset{H}{\underset{H}{C}}{-}\underset{H}{N}{-}\boxed{H+HO}OC{-}\overset{H}{\underset{H}{C}}{-}\underset{H}{N}{-}H = HOOC{-}\overset{H}{\underset{H}{C}}{-}\underset{H}{N}{-}\overset{O}{\overset{\|}{C}}{-}\overset{H}{\underset{H}{C}}{-}\underset{H}{N}{-}H + H_2O.}$$

These can be linked in any order so that from three different amino acids—a, b, and c—three different combinations could be made,—abc, acb, and bac. Such very simple combinations are not complex enough to be called proteins but instead are *polypeptids.* Real proteins probably contain hundreds of such acids, which are not only linked together end to end, but may also contain amino acids attached on the side, as illustrated in the accompanying diagram, where amino acids are attached at the places marked R. When one thinks of the combinations possible

$$\mathrm{NH\underset{R}{\underset{|}{CH}}.CO{-}NH.\underset{R}{\underset{|}{CH}}.CO.NH.\underset{R}{\underset{|}{CH}}.CO.NH.\underset{R}{\underset{|}{CH}}.COOH}$$

with such large numbers of units, which may be linked together not only in straight chains but also in rings, it is not surprising that each species of plant and animal has its own specific type of protein and that there may even be individual differences so that every individual organism might have a protein of its own. This has been suggested, and there is evidence in its favor; if it is not true, it is not because of the lack of protein possibilities.

An important evidence of this specificity of proteins is the *precipitin reaction.* It has been found that if serum from a horse is injected into a rabbit in increasingly larger doses, the rabbit will form in its blood a substance known as *precipitin,* a protein related to the globulins. If the precipitin from the rabbit is then added to the blood of the horse *in vitro* a precipitate is formed, which is a compound of the precipitin with the albumin of the horse serum. This precipitin will react only with horse serum or with the serum of an animal *closely related* such as the mule and the ass. In this way it is possible to determine relationships, and much work of this sort has been done. The precipitin produced by human blood will react with that from the anthropoid apes thus establishing another link in the evidence for human evolution. Korinek (1924), Raeder (1924), Saltzmann (1924), and Mez (1926) have all been doing work of this kind with plants and find that the same type of reactions can be obtained with plant proteins. Thus serum from a rabbit which has been injected with yeast extract will precipitate extract from yeast and that from truffles (which are also Ascomycetes) but not from the ordinary mushroom (which is a Basidiomycete). In the seed plants, injections from different regions—root, stem, or leaf—cause the same precipitin reactions. This work has thus opened a whole new field in the determination of plant and animal relationships. It is interesting to note in this connection that legumes which cross-inoculate with the same bacteria (Chap. IX) have the same type of proteins.

If a small amount of a protein (the *sensitizing* dose) is injected into the blood of an animal and then some days later (7 to 30, depending upon the size of the sensitizing dose) another injection is made of the same protein (the *intoxicating* dose), the animal may undergo a severe shock and even die within a few minutes. This *anaphylactic* shock may be brought about when the sensitizing dose is as small as 0.00000005 g. of protein (egg white) and is another indication of the high specificity of proteins. Hay fever is now considered to be a case of anaphylaxis, in which the patient has become sensitized to some individual protein, generally pollen. This anaphylactic reaction has been reported in plants by Lumière and Coutourier, who sensitized leaves and bulbs to horse serum, using 0.01–0.02 c. c. After three weeks 0.2–0.3 c. c. were given to both the sensitized organs and to controls; four days later those that had been sensitized began to wither and die, while the others remained normal.

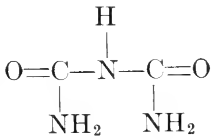
About twenty-five different amino acids have been isolated from proteins, some of which have been found free in plant tissues, particularly in germinating seeds and in rapidly developing buds and shoots, where proteins are undoubtedly undergoing changes. Under these conditions the amino acids are found as transition products in the synthesis or decomposition of proteins. Among the common amino acids are leucine, lysine, arginine, tyrosine, proline, histidine, and tryptophane. (The name of an amino acid generally ends in *ine.*) Different amino acids seem to be connected with different physiological activities in the animal organism. Tryptophane, while essential to the maintenance of life, does not promote growth. Lysine, on the contrary, definitely favors growth. Arginine is associated with reproduction, and cystine with the production of hair, feathers, etc. Such correlations between the amino acids and physiological activities have not yet been noted for plants.

Precipitation Reactions.—Owing to the amphoteric nature of proteins they form insoluble salts and are precipitated out of solution by the salts of the heavy metals such as iron acetate, copper sulphate, etc., which form with the protein an insoluble precipitate. For this reason egg white is administered in cases of poisoning with metal salts like mercury. This differs from the precipitation out of solution by the ordinary saturated salt solutions in that, with the heavy metals, a definite insoluble salt is formed and the protein is altered or denatured.

Color Reactions.—Certain groups or radicals in the protein molecule combine with various reagents to give specific color reactions. The presence of these reactions then determines whether this particular radical or grouping is present in the molecule. The reactions are therefore really tests for certain amino-acid groups rather than for proteins as a whole. Among the several color reactions three are commonly used:

1. *Millon's reaction.* A solution of mercuric nitrate with free nitrous acid present, produces a reddish precipitate in the presence of tyrosin or a protein which contains it.

2. *Xanthoproteic reaction.* Proteins containing tyrosin are turned yellow by nitric acid. This color is intensified by heating and is changed to an orange color on the addition of ammonia. This is the familiar yellow color which develops on the fingers of a laboratory worker who has been handling nitric acid.

3. *Biuret reaction.* If a solution of copper sulphate is added to an alkaline solution of a protein which contains two $CO.NH_2$ groups as in biuret,  $\mathrm{O{=}\underset{NH_2}{C}{-}\overset{H}{N}{-}\underset{NH_2}{C}{=}O}$, a bluish-violet or lavender color results. Since most proteins contain such groups, the biuret reaction can be used for them.

Physical Properties—Indiffusibility.—The proteins belong to the class of bodies known as colloids (Chap. II). Although there are some who think that the individual protein molecules are not so very large but that they group together to form large colloidal complexes, the majority of workers hold that the molecules are of enormous size, being the largest molecules known. Some of the molecular weights have been determined and then from their percentage composition the formulæ have been calculated. The following calculations for typical proteins give some conception of their size and complexity.

Protein	*Source*	*Formula*
Albumin	eggs	$C_{696}H_{1125}N_{175}O_{220}S_8$
Gliadin	wheat	$C_{685}H_{1068}N_{196}O_{211}S_5$
Zein	corn	$C_{736}H_{1161}N_{184}O_{208}S_3$

These are the formulæ for *simple* proteins; others like the conjugated proteins are much more complex.

Since the molecules are so large, the passing of protein through the pores in a parchment or animal membrane is prohibited, although some of the derived proteins (albumoses and peptones), which are obtained by partly breaking down the complex proteins, may pass through such membranes.

Coagulation.—When in a colloidal solution, the natural or native proteins may be turned into a semisolid gel by heat, by alcohol, or by certain enzymes known as *coagulases.* As an example of the first named may be cited the coagulation of an egg during boiling or frying; and the coagulation by enzymes is utilized in the manufacture of cheese, during which the protein is coagulated by the enzyme in rennet. The action is not reversible and the protein cannot be made soluble again unless decomposed into simpler substances.

Optical Activity.—All solutions of plant and animal proteins are optically active and rotate the plane of polarized light to the left.

Precipitation without Change.—In strong salt solutions of the ordinary alkaline earths such as $NaCl$, $CaCl_2$, Na_2SO_4, etc., the proteins are precipitated out of solution, which simply means that the proteins are not soluble in such solutions. Whether this is due to the fact that the salts in some way rob the protein of water and thus precipitate it or to an electrical effect of the salt is not known. The first hypothesis assumes that the salt holds the water from the protein and thus precipitates it, while the second hypothesis assumes that the proteins are held in a colloidal solution because they are mutually repelled by the similar electric charges which they carry. If the salt neutralizes these charges, the force holding the molecules in solution is no longer operative and they settle out.

This type of precipitation differs from chemical precipitation in that the proteins are unchanged and do not combine with the salt. It also differs from coagulation by heat in that here mere dilution of the salt solution will bring the proteins back into solution again; but when coagulated by heat the protein must be decomposed before it will redissolve.

The precipitation by salts is important physiologically because of its connection with cold injury to plants. When water gets down to the freezing point the proteins of the protoplasm are not injured. As the temperature falls still farther the water in the cell sap may crystallize out, leaving the salt in solution and ultimately resulting in the production of a saturated salt solution within the cell. As this condition approaches, the proteins in solution may be precipitated, with the result that the cell dies if this condition persists for a long time. If the precipitated condition has not lasted too long, raising the temperature will restore the proteins to their original state and the cell protoplasm to its normal function.

In *Begonia* the soluble proteins are precipitated when the temperature reaches −3° C. while in the pine, according to Gorke (1906), a temperature of −40° is required to obtain a similar result. This is probably due to the difference in the amount of soluble salts and sugars in the two plants. Plants which are subject to periodic drought like many conifers are said to possess only small amounts of solutes in their sap.

Classification of Proteins.—Proteins were originally classified upon the basis of their solubility, coagulability, and precipitability. Since more has been learned of their chemical characteristics, these are used in addition to the physical characters for classification but, in general, the consideration of chemical characters has not required many important changes in the original classification based upon physical characters. The following is the scheme generally adopted in America, but it differs in some particulars from that used by English biochemists:

I. Simple proteins.
   1. albumins.
   2. globulins.
   3. glutelins.
   4. prolamins or gliadins.
   5. histones.
   6. protamines.
   7. albuminoids or scleroproteins.

II. Conjugated proteins.
   1. chromoproteins.
   2. nucleoproteins.
   3. glucoproteins.
   4. phosphoproteins.

III. Derived proteins.
   A. Primary derivatives.
      1. metaproteins.
      2. coagulated proteins.
   B. Secondary derivatives.
      1. proteoses or albumoses.
      2. peptones.
      3. peptids.

Simple Proteins.—The simple proteins yield only amino acids when hydrolyzed. The *albumins* are soluble in water and dilute salt solutions and are coagulated by heat. They are widely distributed in plants and animals. Egg albumin is the most familiar animal albumin, while among the plant albumins are leucosin (wheat, rye, and barley), legumelin (legume seeds), and ricin (castor beans).

Globulins are insoluble in water, soluble in dilute salt solutions, and are coagulated by heat. The vegetable globulins form the major portion of the protein of most seeds except the cereals and are among the most common protein reserves. Among the common globulins are legumin (beans and peas), amandin (seeds of almonds, peaches, and plums), acerin (maple), and edestin (hemp). Fibrinogen from the blood is a familiar animal globulin.

Glutelins are insoluble in water and dilute salt solutions but are soluble in dilute acids and alkalies. They are not coagulated by heat. No animal glutelins have thus far been isolated and only a few from plants. These are in the cereals,—glutenin (wheat) and oryzenin (rice).

Prolamins or *gliadins* are insoluble in water and salt solutions but are soluble in dilute acids and alkalies as well as in 80% alcohol. This solubility in alcohol is their outstanding characteristic. Thus far they have been found only in the plant kingdom and there only in the cereals. Among the better known prolamins are gliadin (wheat and rye), hordein (barley), and zein (corn). Gliadin has the peculiar property of combining with water to form a sticky paste which holds the particles of glutenin together; the two, glutenin and gliadin, form *gluten*, which gives the sticky and elastic properties to dough made from wheat flour.

The *histones*, which are soluble in water, insoluble in ammonia, and are not coagulated by heat, have not yet been found in plants.

The *protamines* are the simplest proteins known. They are soluble in water and ammonia and are not coagulated by heat. They are related to the histones but are simpler. None have been found in plants although there is evidence that they may occur in pollen. In fish sperm, several protamines have been found.

The *albuminoids* are insoluble in water, salt solutions, acid, or alkalies. They have not been found in plants but include such animal proteins as keratin (hair and feathers) and gelatin (hoofs and horns).

Conjugated Proteins.—The conjugated proteins yield a true protein when hydrolyzed and, in addition, some other substance of a quite different nature. They are rarely found in plants and some groups are represented by only a few members. The *chromoproteins* are combinations of protein with a pigment. Hemoglobin is such a protein, and the special pigments of the algæ also belong here (Chap. XV).

The mucilage from the root of the Japanese yam (*Dioscorea*) is believed to be an example of *glucoproteins*, which are compounds of protein with carbohydrates. The *nucleoproteins* are compounds of nucleic acid with proteins and are the chief constituents of the nuclei. They have been found by Osborne (1909) in small amounts in the embryos of seeds, but whether these proteins occur free there or are produced by the methods employed in their isolation is uncertain.

The *phosphoproteins* such as zymocasein in yeast are rarely present in plants. They yield a protein and phosphoric acid, thus bearing somewhat the same relation to the proteins that the phospholipins do to the fats. The two substances are commonly associated, e. g., in egg yolk, cheese, etc. Combinations of proteins with phospholipins also exist and are known as *lecithoproteins*, which are therefore a kind of phosphoprotein.

Derived Proteins.—The derived proteins include decomposition products which are obtained from simple proteins by hydrolysis or coagulation. They are transition products and do not occur as permanent forms in the tissues.

The *metaproteins* are the early products of hydrolysis by dilute acids or alkalies. They are insoluble in water or salt solutions but do dissolve in dilute acid and alkali. The *coagulated proteins* are also primary derivatives which are produced by the coagulating action of heat or alcohol.

The *proteoses* or *albumoses* are produced from the simple proteins by the action of hydrolytic agents such as the enzymes, pepsin and trypsin. They are soluble in water, salt solutions, dilute acids, and alkalies, but are not soluble in ammonium sulphate solutions. As found in almonds and wheat they give the reactions for proteins but are not coagulated by heat. This is the chief difference between the albumoses and the albumins.

The *peptones* are further decomposition products which still give the protein reactions including that of the biuret. They are not precipitated by saturated solutions of ammonium sulphate and are unique among proteins in this respect. The *peptids* are the last products formed before the amino-acid stage is reached. They are made up of small numbers of amino acids and may or may not give a biuret reaction.

Comparison of Plant and Animal Proteins.—Animals contain a greater variety of proteins than plants. Of the simple proteins, three groups—histones, protamines, and albuminoids—have not yet been found in plants, while the conjugated proteins are rare. This difference is partly counterbalanced by the presence of gliadins and glutelins only in plants. The fact that animals contain a greater variety of proteins is associated with the difference in the lives of the two groups of organisms and the wider variety in the diet of animals. It should also be added that they have been much more thoroughly studied.

Of the two groups of simple proteins found in both plants and animals, viz., the albumins and globulins, it is noticeable that the plant proteins are often more easily precipitated by salt solutions and are less easily coagulated by heat than their animal analogues.

Plant proteins in general are a bit higher in nitrogen than animal proteins, with the exception of the protamines. This is probably due to their higher content in histidine and arginine, amino acids rich in nitrogen. Of some twenty-five simple plant proteins thus far investigated, about 10% are glutelins, 13% gliadins, 17% albumins, and 60% globulins.

Complete Proteins.—From the point of view of nutrition, a protein is considered a complete protein if it contains the necessary variety of amino acids not only to support life but to permit growth and reproduction. Such complete proteins are rare, and few plant proteins are complete for animal nutrition. Zein, e. g., contains no glycine, lysine, or tryptophane and would not support animal life if used as the only source of protein food. Gelatin, which lacks cystine, tyrosine, and tryptophane, is another example of an incomplete protein. This is only one of the reasons why a wide variety of foods in the diet is beneficial.

Although animals may be fed amino acids instead of proteins, glycine is generally considered the only one which animals can synthesize from the others. Recently some doubt has been thrown upon the generality of this statement in connection with cystine, which is the chief amino acid associated with sulphur and the one through which this element enters the protein molecule. Keratin, the chief protein in wool, is 13% cystine; and it has been estimated that a sheep, in order to get the necessary cystine, would have to eat about 60 pounds of fresh grass each day! As a tentative solution to this problem, it has been suggested that perhaps the cystine is synthesized somewhat by the microörganisms in the stomach of the sheep. Or does the sheep really synthesize its own cystine? Whatever the answer may be, we here see some of the interesting sidelights of the questions involved.

Synthesis of Proteins.—Concerning the formation of proteins there are several different theories, the chief of which may be classified as follows:

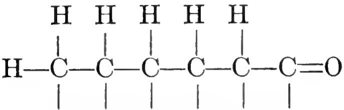
1. That they are made from amino acids
    a. which come from carbohydrates.
    b. which do not come from carbohydrates.
2. That they are made from some other nitrogen compound.

The fact that as high as eighteen simple amino acids have been linked together in the laboratory by Emil Fischer (1901) to form simple proteins or peptids, coupled with the fact that when hydrolyzed the proteins yield amino acids, would almost settle this question. In truth, since the days of Fischer this origin of the proteins is quite generally accepted. The amino acids are built up into proteins by means of enzymes, which can, under the proper conditions, also hydrolyze the protein back to its constituent amino acids. The proteins are thus seen to be comparable to the polysaccharides. As the latter are made up of groups of monosaccharides, in like manner the proteins are built up of amino-acid units. Zaleski (1911) showed that in the ripening of peas the protein increased at the expense of the amino acids and the other nitrogen compounds present.

Time	*Per cent proteins*	*Per cent amino acids*	*Per cent N bases*	*Per cent other N compounds*
At beginning	79.2	8.7	10.8	1.4
Five days later	89.2	4.6	5.6	0.8

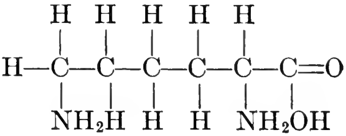
During germination there is a similar diminution of the protein and an increase of the amino acids present. The problem then becomes one of the origin of the amino acids.

Whether these are made from carbohydrates or not is a much disputed point. If we compare the structure of glucose with that of the amino acid, lysine, a marked similarity is observed.



$$H-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-\underset{OH}{\overset{H}{C}}-\underset{H}{C}=O$$

glucose



$$H-\underset{NH_2}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{NH_2}{\overset{H}{C}}-\underset{OH}{C}=O$$

lysine

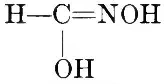
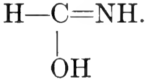
After the glucose was once changed to a fatty acid, the difficulties of which were considered in the last chapter, the change to the amino acid would not be so hard to accomplish. It has been thought for some time that the plant combined the nitrogen from nitrates with the transformed carbohydrate and thus produced the amino acid. It was even thought that this might be dependent upon light since proteins are made ordinarily only in the presence of light. In the dark, nitrates collect in the plant, but disappear in the light. Zaleski and Suzuki (1897–1901) showed, however, that if sunflower leaves were floated upon a solution of sugar and nitrate, much protein was manufactured even in the dark. Palladin (1899) obtained similar results with bean leaves, but even here more protein was manufactured in the light. Leaves which contained 18.6 g. of protein when gathered, contained 67.2 g. at the end of six days in the dark on a sugar solution, and 140.9 g. in the light. The light, however, seems necessary chiefly for the production of the preliminary carbohydrates, which explains why the nitrates collect in the plant in the dark. If plenty of carbohydrates are available from outside, as in the experiments of Zaleski and Suzuki, the protein manufacture will continue in the dark.

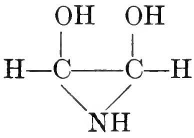
In the laboratory, amino acids have been synthesized by the action of ammonia on glyoxylic and sorbic acids, both of which are found in plants and may be obtained from the oxidation of simple sugars. Does the plant use this same method? The answer is doubtful.

It has been suggested that the light plays a prominent part in the synthesis of proteins not only in the manufacture of carbohydrates but in the transformation of nitrogen from the nitrate into the amino form. From this angle the problem was attacked by Baudisch (1911–1918) who found that when a mixture of potassium nitrite and methyl alcohol in water was exposed to ultra-violet light, the methyl alcohol was oxidized to formaldehyde at the expense of oxygen from the nitrite, which (as hyponitrite) then reacted with the formaldehyde to produce the potassium salt of formhydroxamic acid. This reaction is purely photochemical and cannot be made to take place in the dark even by boiling:

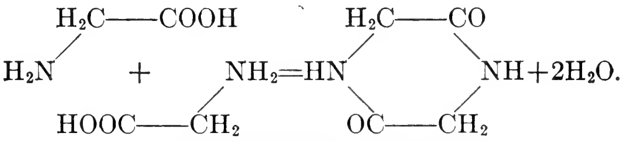
$$KNO_2+CH_3OH=KNO+HCHO+H_2O$$

$$KNO+HCHO=H.CNOK.OH.$$

Baly and others (1922) have confirmed these results and have found that similar results may be obtained starting with nitrates and carbon dioxide. Under the action of the ultra-violet light the carbonic acid is transformed into activated formaldehyde which results in the potassium formhydroxamate as found by Baudisch. This potassium salt is hydrolyzed to the free acid  $\mathrm{H{-}\underset{\underset{OH}{|}}{C}{=}NOH}$ and then reduced to  $\mathrm{H{-}\underset{\underset{OH}{|}}{C}{=}NH}$. This compound, they believe, then condenses with more of the formaldehyde to form a labile ring compound



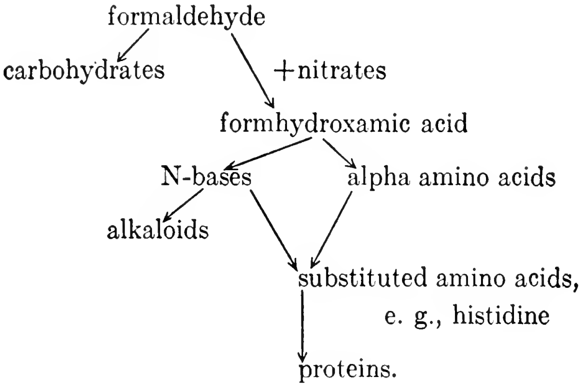
which is rearranged to form glycine CH_2NH_2COOH, a simple alpha amino acid. These amino acids may then be built up into proteins according to Shibata (1926) as follows:



2 amino acids = glycine anhydride + water

Further condensations result in the complex proteins found in the plant.

It is to be noted that these authors conclude that (1) protein synthesis is dependent upon light only for the formation of the active formaldehyde, (2) part of the formaldehyde is used for making proteins and part for the carbohydrates as described in Chapter VI, and (3) part of the formhydroxamic acid is used also for making the nitrogen bases and the alkaloids, both of which are considered to be waste products. This entire scheme may be represented by the following diagram-summary:



The chief objections to this work of Baly are that (1) it is based entirely upon work *in vitro*, and (2) it has been shown rather conclusively that light is not necessary for the synthesis of proteins aside from the question of the carbohydrate supply.

The main steps in the general process are now fairly well agreed upon. The nitrates, as stated above, are combined with carbohydrates or their derivatives to form the amino acids, which are then combined into proteins. It is a curious fact that although most plants can use nitrates better than ammonium compounds (Chap. IX), the plant must first transform the nitrates into nitrites and then into ammonia by the aid of reducing enzymes called reducases (Eckerson) in order to produce the amino form of nitrogen necessary for the production of amino acids.

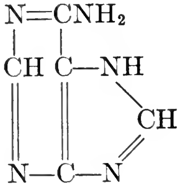
Animals are rather reckless in their use of proteins. Since they get their supply of this element from the outside by feeding on inferior forms, they have not been forced to conserve their nitrogen supply as have plants. The result is that large amounts of nitrogenous wastes are excreted daily in the form of urea. Plants do not excrete nitrogen in this fashion but use the same materials over and over. The proteins inside the plant are broken down to ammonia compounds and then reused the same as the nitrogen that comes in through the roots or leaves (in insectivorous plants). But this material is not broken down into ammonia for amino acids except when needed, but instead is stored (also translocated, according to some students) in the plant as the compound called *asparagine,* which plays a very special rôle in the protein economy of plants. Although the ultimate waste product, corresponding to urea in animals, it is not excreted but is stored until the time for it to be used, when it is broken down into ammonia and starts again on the internal nitrogen cycle of the plant. This may be diagrammed as follows:

$$\begin{tikzcd}
 & \text{amino acids} \arrow[r] & \text{proteins} \arrow[r] & \text{amino acids} \arrow[d] \\
HNO_3 \arrow[r] & NH_3 \arrow[u] & \text{asparagine} \arrow[l] & NH_3. \arrow[l]
\end{tikzcd}$$

One may ask why NH_3 is formed *before* the asparagine, when the amino acids are broken down. The reason for this is that not all proteins yield asparagine directly, but the asparagine must be synthesized from its components,—an amino acid (aspartic) and ammonia. As Prianishnikov has stated it: "Ammonia is the primary product and at the same time the end-product of the transformations of nitrogenous substances within the plant."

We have thus seen the general steps in protein synthesis and metabolism, but the details of the process are still among the trade secrets of the plant. In some of the lower plants, notably the fungi, urea plays somewhat the same rôle as asparagine does among the higher plants. Here again one is impressed with the fact that the fungi resemble animals in many of their metabolic aspects more than they do green (autotrophic) plants, although it should also be noted that the urea is a reserve food for the fungi and not a waste product (as in animals).

Treub's theory (1895–1907) of the synthesis of proteins from hydrocyanic acid should be mentioned although it has few followers at the present time. Many common plants have glucosides which contain hydrocyanic acid (Chap. XV). When left in darkness these disappear, and form again when exposed to light if the plant is supplied with nitrates. Treub thought that the HCN polymerizes into substances like adenine,



which are then built up into proteins. There is little in support of this theory and it is questionable whether free HCN is ever found in plants. The formation of the glucosides in light is probably due to the formation of the glucose under those conditions, and the combination with the HCN may be a way of removing poisonous waste products which result from some other activity within the plant. Rosenthaler (1927) found an increase in HCN in *Sambucus* when amino acids were supplied to the plant, and suggested that the HCN production occurs when plants produce or contain an excess of amino acids. The HCN is considered not exactly a "waste product" but rather an auxiliary form of nitrogen metabolism resorted to under these conditions. It thus seems to be of only minor importance in the large problem of protein synthesis.

QUESTIONS

1. Why are legumes generally richer in proteins than other plants?
2. Why do growing organisms need more protein than mature ones?
3. What is the chief function of protein within the plant?
4. Discuss the life and work of Emil Fischer.
5. Are the color reactions infallible tests for proteins? Why?

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CHAPTER XV

ALLIED AND MISCELLANEOUS PRODUCTS

> Variety's the very spice of life,
> That gives it all its flavor.
> —WM. COWPER.

In the plant factory, aside from the foods mentioned in the three preceding chapters, are made other products, which are more or less closely related and which have certain physiological rôles in the plant. These will be described in the following order:

1. Glucosides.
2. Organic acids.
3. Nitrogen bases.
4. Aromatic aldehydes, alcohols, phenols, and acids.
5. Essential oils and resins.
6. Tannins.
7. Pigments.

Glucosides.—The glucosides are compounds of sugars with various other substances usually belonging to the class of aromatic compounds. Strictly speaking they should always contain *glucose*, but the term is rather a loose one which includes many different substances, and in some cases the sugar present is galactose, mannose, or levulose. Even combinations with pentoses are not unknown. To include the different substances found here under one class is to a certain extent artificial and, if the compound associated with the sugar is more important than the sugar, the substance may more fittingly be placed in another group, as in the case of the glucoside pigments.

The glucosides are generally soluble in water, forming a levorotary solution. When purified they form colorless crystalline solids with a bitter taste. They are hydrolyzed by specific enzymes. Several hundred glucosides have been isolated from plant tissues, and among the products resulting from their hydrolysis, aside from sugars, are alcohols, aldehydes, hydrocyanic acid, phenols, mustard oil, saponins, flavones, anthocyans, etc. The glucosides are classified upon the basis of these products.

Among the alcohol glucosides is salicin from willow bark and leaves. It is used in medicine for the treatment of fevers and rheumatism, and when hydrolyzed it breaks down into salicylic alcohol (saligenin) and glucose:

$$C_{13}H_{18}O_7+H_2O=C_7H_8O_2+CH_{12}O_6.$$

The cyanophore glucosides yield HCN as one of their products. The most common glucoside in this group is amygdalin, which was one of the first glucosides isolated and which has probably been studied more than any other. It has commercial value as the essential material in oil of bitter almonds, which is much used as a flavoring extract. When hydrolyzed by the enzyme, emulsin, it breaks down into benzaldehyde, hydrocyanic acid, and glucose:

$$C_{20}H_{27}NO_{11}+2H_2O=2C_6H_{12}O_6+HCN+C_6H_5CHO.$$

It occurs in almonds, in the seeds of apples, peaches, and plums as well as in the leaves of cherries, peaches, and the cherry laurel, where it is often the cause of stock poisoning as a result of the liberated HCN. The enzyme and glucoside are evidently kept apart in the cell under normal conditions and not till the cells are crushed and eaten does the hydrolysis result.

Among the mustard-oil glucosides may be mentioned sinigrin, which occurs in mustard, horse-radish, etc. When hydrolyzed by the enzyme, myrosin, it is broken down into glucose, mustard oil, and acid potassium sulphate:

$$C_{10}H_{16}O_9NS_2K+H_2O=C_3H_5NCS+C_6H_{12}O_6+KHSO_4.$$

The saponins form another important group of glucosides. They are amorphous, colloidal substances which produce a soapy froth when dissolved in water. When shaken with fats and oils they produce a stable emulsion, enabling them to be used as soap substitutes, and it is from this property that the name saponin comes. The watery solutions of saponin also have the property of dissolving large quantities of gases such as carbon dioxide, and they are occasionally used in soft drinks for this purpose, although this should be discouraged because the saponins are toxic, producing hemolysis (dissolution of the red corpuscles). Fish are especially sensitive to such toxins and are killed by a solution of one part in a hundred thousand. In certain regions of the East saponins are used for this purpose. They have been found in several hundred plants scattered throughout the plant kingdom and may be universal in small amounts. When hydrolyzed they yield a hexose and another substance known as a sapogenin (saponin former). This may be illustrated by the hydrolysis of digitonin from *Digitalis* which yields digitogenin, glucose, and galactose:

$$\underset{\text{digitonin}}{C_{54}H_{92}O_{28}}+2H_2O=\underset{\text{digitogenin}}{C_{30}H_{48}O_6}+\underset{\text{glucose}}{2C_6H_{12}O_6}+\underset{\text{galactose}}{2C_6H_{12}O_6}.$$

The flavone and anthocyan glucosides may be better considered with the pigments.

Physiological Significance of Glucosides.—With so many different kinds of glucosides it is not reasonable to expect that all will have the same function in the plant. This wide variety of substances undoubtedly has an equally wide variety of functions, among which may be mentioned the following:

1. They act as sources of reserve food because of the sugars they contain. Their occurrence especially in seeds lends support to this view. In bitter almonds the glucoside is hydrolyzed during germination, when the free HCN can be detected; and in *Prunus laurocerasus*, during periods of starvation these glucosides disappear. The increase of the glucosides during periods of photosynthesis also points toward this function. In the willow, salicin is formed during the day and is split up at night, according to Weevers (1910). The saligenin remains in the leaf to be used the next day, while the glucose is translocated to other parts of the plant. Not only is there a daily variation in the amount of glucoside but a seasonal one as well. In *Salix*, *Populus*, *Taxus*, and *Vaccinium* the glucoside is most abundant in the fall and winter, dropping to its lowest levels in the spring when the new shoots form.

2. Guignard (1906) does not think that the cyanophore glucosides can be used as reserve foods because of the toxic action of the HCN, but inclines rather to the view that these glucosides fix injurious by-products either permanently or temporarily in this form. The occurrence of glucosides in young shoots and leaves where metabolism is active is explained on this basis, and the large percentage of glucosides in some plants during unfavorable conditions gives support to this waste-product theory.

3. Peche (1912) thinks the glucosides are direct products of photosynthesis. Some are stored for future use while others, more labile, are quickly broken down. The relation of these products

to Treub's theory of protein formation has been previously mentioned.

4. In the base of extrafloral nectaries as well as in the surrounding tissue, are commonly present glucosides, which may play a part in the secretory action of the nectaries.

5. Easily oxidizable materials such as aldehydes and acids may be protected against too rapid or premature oxidation by being transformed into glucosides. This has been given as an explanation of the aldehyde and acid glucosides.

6. Many anæsthetics like ether and chloroform have a marked effect upon growth (Chap. XXVI), behaving like a stimulant, and in many cases the rapid growth which results is accompanied by the hydrolysis of glucosides present. It has been suggested that the rapid growth is due to a stimulus provided by some component of the glucoside, or that the glucoside contains one of the plant stimulants to which the name *hormone* has been given (Chap. XVII). This occurrence of glucosides in rapidly growing tissues was mentioned above (2), but according to the conception there presented the presence of the glucosides was explained in an entirely different way.

7. The bitter taste and poisonous nature of many glucosides may serve as a protection against herbivorous and fructivorous animals. In seeds, leaves, and bark such protection would be very helpful, and there is no doubt that the presence of such substances does render the parts which contain them less attractive.

8. Many of the nonsugar elements of glucosides are bactericidal and prevent the growth of fungi and bacteria. Their presence in bark provides a naturally antiseptic condition, which may be useful to the plant in case of wounds, and it may well be that one of the reasons why the processes of decay do not easily set in until the cell is dead, is because of the presence of these antiseptic glucosides. It is interesting to recall in this connection that the glucoside is not normally hydrolyzed in leaves, seeds, etc., until the tissue is wounded or cut. This permits the enzyme to react on the glucoside with the production of the toxic or antiseptic compounds.

Organic Acids.—Aside from the organic acids associated with the fats, other acids are often present in the cells of plant tissues, where they are formed by the incomplete oxidation of carbohydrates under certain, special conditions. Among those not previously mentioned are acetic, malic, oxalic, tartaric, and citric acids.

Acetic and lactic acids are produced in the fermentation of carbohydrates by bacteria and are the familiar acids in ensilage and sauerkraut.

Oxalic acid is found free in sorrel (*Rumex*), *Oxalis*, and rhubarb (*Rheum*). In many other plants it occurs as a salt of calcium and potassium. The calcium oxalate crystals which are often found in plant cells are insoluble, and this is thought to be a method of getting rid of the excess calcium.

Malic acid is found in apples, cherries, and many small fruits as well as in many vegetables. In boiling down maple syrup, calcium malate is found in the bottom of the vats as "sand."

Tartaric acid is found in many fruits but especially in grapes. In the fermenting of grape juice to make wine, large quantities of the monopotassium salt of tartaric acid precipitate out, from which is prepared the commercial "cream of tartar" used in baking.

Citric acid is found especially in the citrus fruits, but it also occurs in many small fruits like loganberries, cherries, and currants, where it is associated with malic acid.

These acids perform important functions:

1. They regulate the acidity of the cell sap and thus of the medium in which much of the work of the cell is carried on. All reactions, notably those of enzymes, have an optimum acidity; and the acids serve to determine this within the cells where they are found. They may thus regulate the aggregation of colloids and other colloidal phenomena. The amount of acid in a tissue may change with the state of development of the tissue. As fruit ripens the amount of acid generally diminishes. Seeds generally contain small amounts of acid which increase as the seed germinates. According to this conception, there is no reason why different plants should produce different acids if their function is merely to insure a favorable environment for the chemical processes which take place in the growing plant, including the conversion of reserve food into an available form and its translocation.

2. They serve to make certain ripe fruits more attractive by their pleasant odors and flavors and thus aid in the distribution of the species.

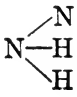
3. In green fruits their presence may make the fruit unpalatable and unattractive so that it will not be touched by distributing animals before the seeds are mature.

4. In ferns, the sperms are attracted to the female gamete by malic acid produced by the archegonium.

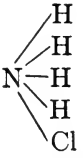
5. They may serve as a sort of reserve food or temporary storage product. The calcium oxalate crystals in *Sterculia platanifolia* dissolve at blooming time, and starch appears in the cells which contained the crystals, which here seem to be reserve foods rather than excretory products.

That the acids are associated with carbohydrates, from which they probably arise is shown by the fact that the amount of acid present depends in a general fashion upon the amount of carbohydrate; and plants grown under "long day" conditions, i. e., which produce more carbohydrates, contain more acids than plants illuminated for only a short period daily. In *Phaseolus* and *Sempervivum,* however, at 35° and in light, not so much acid was found as in the dark or at 5°, i. e., in conditions favorable for producing carbohydrate, less acid was found.

Nitrogen Bases.—The vegetable bases all contain nitrogen and may be considered as derivatives of ammonia, which can form salts by combining with acids and thus shows its basic nature. The fact that nitrogen may be pentavalent and combine with five different kinds of radicals increases the number of basic compounds which in this way can be derived by substituting other radicals in the ammonia for the hydrogen atoms.



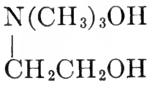
ammonia



ammonium chloride

On the basis of their structure the plant bases may be divided into three groups,—the natural bases, the alkaloids, and the purine bases.

Natural Bases.—The natural bases or amines are open-chain compounds with substituted radicals in the ammonia as described above. Among these are choline and muscarine, which occurs in the poisonous mushroom, *Amanita;* but they are also found in fruits, seeds, roots, etc. Choline has the formula



$$\begin{array}{l} N(CH_3)_3OH \\ | \\ CH_2CH_2OH \end{array}$$

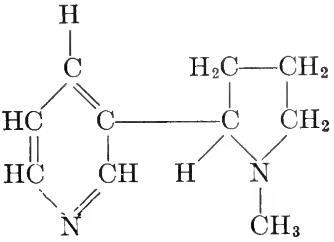
which typifies this class of compounds and shows its relation to ammonia. Among the natural bases are the so-called "ptomaines," which are decomposition products of proteins (fish, meat, etc.) produced by the fermentations of bacteria. Some of them are very toxic.

There is no known use for the natural bases. Some investigators consider them as intermediate products in the formation of proteins, but this does not seem likely or they would be more widely distributed. It is more reasonable to suppose that they are intermediate products of protein metabolism under abnormal circumstances, e. g., insufficient oxygen or lack of carbohydrates. Where stored in quantities, as in certain mushrooms, they may be a source of nitrogen reserve.

Alkaloids.—These compounds are not widely distributed in plants but are associated with certain families such as the Solanaceæ (nightshades) and Papaveraceæ (poppies). They are generally colorless, odorless, crystalline solids, which are insoluble in water but easily soluble in the common organic solvents. The salts of some alkaloids such as nicotine and quinine sulphates are, however, also soluble in water. Most of them have a decided bitter taste and possess marked physiological or toxic properties, which make them important in pharmacology and medicine. Unlike the natural bases they are "aromatic" compounds, i. e., the carbon atoms are in a ring and not in an open chain.

Their origin in the plant is in some dispute. Pictet (1907) believes they come from the condensation of simple nitrogen compounds, which have arisen from the breaking down of complex forms such as proteins. Baly (Chap. XIV) has proposed that they come from the nitrogen bases parallel with the construction of proteins and not subsequent to it.

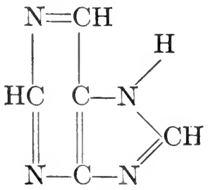
Among the well-known alkaloids are nicotine (tobacco), atropine (nightshade), cocaine (coca), quinine (cinchona), and morphine (poppy). The formula for nicotine will serve as an example of the group structure.



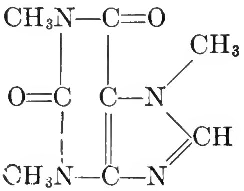
Whether the alkaloids are waste products from nitrogenous materials or are direct synthetic products has not been determined. According to Annett (1920), the morphine alkaloids are waste products of the poppy and the latex system serves to get rid of them. Baly thinks the alkaloids are waste materials which tie up the waste nitrogen in an insoluble and harmless form. Certainly they are not necessary for the plant which bears them, as is shown by Lotsy (1900) in the case of the cinchona tree, which thrives well in hothouses but makes no quinine. Certain of the Thallophytes can use alkaloids as food if in a dilute state. According to Comère (1910) fungi have been found to use small amounts of morphine, and certain algæ have been able to use atropine, cocaine, and morphine but not quinine or strychnine. Calutriau (1900) reports that higher plants cannot use alkaloids as the sole source of nitrogen. Although plants produce more alkaloids in the sunlight than in the shade (Goris and Deluard report *twice* as much in *Atropa belladonna*), this offers no clue to their function. Whether useful in protein synthesis or merely waste products therefrom, one would expect more to be formed in the sunlight.

In small amounts, alkaloids stimulate the germination of seed and the development of seedlings, according to Longo and Paderi (1929), who concluded that alkaloids might serve as regulators and stimulators of metabolism. On the other hand, nicotine has been found toxic to the germination of tobacco seed; and some workers see in the toxic nature of alkaloids their chief survival value. They are thus considered to protect the plants against destructive enemies such as browsing animals and insect pests.

The Purine Bases.—These are all derivatives of the same substance, purine ($C_5H_4N_4$). Adenine (wheat, beets, tea, and clover), caffeine (tea and coffee), and theobromine (cocoa) are among the better known purine bases found in plants. If the structural formulæ of caffeine and purine are compared, the relationship will be evident.

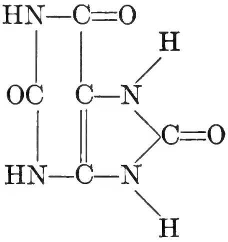


purine



caffeine

As waste products the purine bases seem to answer the requirements better than the others. Uric acid which is found in the urine of animals has the formula:

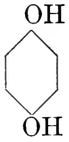
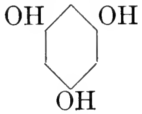


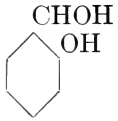
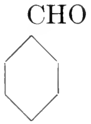
It is thus seen to be a purine base, showing that there is more than a superficial resemblance between urine and tea. The uric acid is known to be an excretory product, and it is only reasonable to suppose that the similar compounds in plants play an analogous rôle. Plants have no method of active excretion for eliminating nitrogen wastes, and these purine bases may in certain cases meet this metabolic demand, the nitrogen being stored in an insoluble manner which cannot harm the plant.

Weevers (1930), however, holds that these purine bases, or xanthene derivatives as they are frequently called, function in much the same way as asparagine (see preceding chapter), i. e., they are formed from the breaking down of proteins and are then used again to make new proteins. They are thus both "waste" and "feed" material, depending upon the phase emphasized. Weevers bases this theory largely on the fact that in plants rich in these products, e. g., tea, coffee, cocoa, herba mata, etc., when branches or leaves die, the xanthene derivatives are not permitted to fall off with the dead organs but are transformed and sent back into the trunk or other active, living parts of the plant. As we have seen, plants are very saving of their nitrogen, and this theory deserves serious attention. Theron and Cutler (1924) believe the alkaloids play a somewhat similar rôle when present and cite a similar group of facts to prove their point.

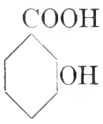
All of the plant bases may be of some survival value in protecting plants against the attacks of animals and fungi and many of them, e. g., the decomposition products of the glucosides, have an antiseptic property.

Aromatic Phenols, Alcohols, Aldehydes, and Acids.—In this heterogeneous group of compounds are placed those with the carbons in a ring. That is, they belong to the *aromatic* series as classified by the organic chemist.

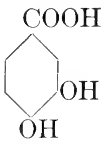
Phenols.—The phenols are aromatic hydroxides as shown by the formula for hydroquinone:  which is the only one with two OH groups that is found in plants in the free state. It is found in the leaves of the cranberry and also as a glucoside in many of the heaths. Phloroglucin,  with three hydroxyls is very widely distributed. Thymol from thyme also belongs in this group.

Aldehydes and Alcohols.—Aromatic alcohols and aldehydes are very common constituents of glucosides. Saligenin or salicylic alcohol from the glucoside, salicin, has the formula:  while benzaldehyde (from amygdalin)  and cinnamon oil are examples of aromatic aldehydes. Here also might be placed the sterols, but because of their relation to the fats, they were discussed in Chapter XIII.

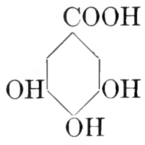
Acids.—Of the aromatic acids the best known are salicylic, protocatechuic, and gallic acids:



salicylic



protocatechuic



gallic

The last two form the basis of the tannins and will be discussed later. Gallic acid occurs in tea and wine and the barks of many trees, including the oak and hemlock.

Essential Oils and Resins.—Associated with these are the essential oils and resins, which are also aromatic compounds. To the essential oils are due most of the characteristic odors of plants. They may have different structures but are similar in their possession of distinctive odors and tastes. The group is thus a loose one which comprises many substances that are not very closely related chemically. Here are the terpenes of which isoprene from rubber is a well-known derivative. Others are menthol (alcohol) and camphor (ketone). Allyl sulphide or garlic oil, found as a glucoside in onions, garlic, radishes, etc., is one of the few essential oils containing sulphur.

The resins are oxidation products of the terpenes. Some are liquid, like Canada balsam and turpentine, while others are very hard like copal and amber (a fossil resin). They are extensively used in varnishes, water proofing materials, etc. Some occur as mixtures with gums, of which asafetida ("fetty") is an example.

What biological significance is possessed by these aromatic compounds can only be guessed at. They are so varied in their chemical nature that it is impossible that they should have the same function. Those which have pleasant odors and tastes may serve to attract animals which aid seed dispersal, and would thus have survival value. It is interesting to note that plants growing in tropical climates, where there is a keen struggle for existence, are more likely to contain such materials, but this may be merely a result of the environment and have no relation to "natural selection." Where the odors or flavors are disagreeable, as in the garlic and onion, they may be explained as protecting the plant against herbivorous animals.

Many oils and resins, such as eucalyptus oil and turpentine, have antiseptic properties and are secreted especially when the plant is wounded. They undoubtedly protect the plant against the entrance of bacteria and other fungi. The redwoods owe their longevity in part to this protective action of the secreted oils, which confer upon the wood its lasting qualities. Creosoting of timber does artificially what the conifers have done for themselves.

Tannins.—The tannins, many of which are glucosides, include a large group of substances that have certain common characteristics. They are amorphous, colloidal, astringent materials, which precipitate gelatin and combine with tissues containing gelatin to form insoluble compounds. For this reason they are used in tanning to convert hides and skins into leather. They all give blue or green compounds with iron salts, and were for this reason used formerly in the manufacture of inks, but in both the tanning and the ink industries the tannins have been largely replaced by synthetic substitutes. The tannins are compounds of protocatechuic and gallic acids, and are divided into two groups on this basis.

Tannins are very widely distributed in nearly all plants, but woody perennials of the temperate zones are especially rich in them. The leaves and bark of the oaks, chestnuts, and sumacs contain large quantities of tannin, which is also found in abundance in unripe fruits and in the pathological growths known as galls. The astringent taste of green persimmons, of poorly prepared tea, and of bad wine, is due to the presence of tannins. Although found throughout the plant, they tend to accumulate in dead regions such as the outer bark.

Many theories have been proposed to explain the presence of the tannins, and since they vary in different species it is not surprising that several ideas concerning their use have been suggested. What may be true for one tannin may not be true for all, and the same tannin may serve different purposes under different conditions.

1. Although the tannins are not a direct photosynthetic product, they are dependent upon light and carbon dioxide for their formation, which is probably the result of their glucoside nature. Oak seeds, when germinated in the dark, show an increase in tannin as the glucosides are hydrolyzed; and the mulberry shows more tannin in the twigs in the morning than at night (Ghirlanda, 1920). The tannins behave in this respect like reserve foods, and this is the function assigned them by many workers, including Michel-Durand, who places in the reserve food category especially those derived from protocatechuic acid.

Although fungi and perhaps a few other plants can use tannin as a source of food in the absence of anything better, the evidence is against the normal use of tannins as a reserve. An argument against their use as reserves is their distribution; they are not found in the sieve tubes or ray cells, where other reserve foods are found, but in the bark and leaves which are cast off. Furthermore, plants rich in tannins, according to Hillhouse (1888), do not draw upon their tannin reserves when grown in the dark as would be expected. In evergreen leaves there is no diminution during the winter when other foods are not being manufactured, although this might be explained by the fact that other reserves are used first. Schell found that the tannin in oily seeds was used only when the oil reserves were gone.

2. The tannins are more likely waste products than reserve foods. The distribution in the leaves and bark agrees with this hypothesis. In rapidly growing leaves and shoots, in growing points, in germinating seeds, and in very irritable regions like pulvini, where the protoplasm is especially active, tannins are found. They are not intermediate products in the formation of proteins but, according to Sachs, are waste products resulting from their decomposition. The glucose is attached to the compound in order to make it more soluble and to facilitate its transport.

3. In the pines, the resin is more abundant in the spring, and the tannins decrease as the resin increases. The cells around the resin canals are also rich in tannin and starch. For these reasons, Wiesner concluded that tannins were an intermediate product in resin formation.

4. Tannins are present in unripe fruits, imparting to them their characteristic astringent taste familiar in green apples and green persimmons. This generally disappears as the fruit ripens, while sugars, the fruit esters which give the specific aromas, and the bright-colored pigments, develop. Some investigators have thought that the tannins give rise to the sugars, esters, and pigments. There is good evidence to support the relation between the fruit pigments and the tannins, and even some of the sugars and esters may arise in this manner; but it has recently been shown that the tannins do not actually disappear in ripe fruits. Instead they are inclosed in special cells surrounded by an insoluble membrane and, as a result, when they are eaten by an animal there is no disagreeable taste. This membrane formation may be hastened by various chemical agents, among which is carbon dioxide gas; and the artificial ripening of persimmons by this method is now a standard commercial process. The walling off of the tannins as the fruit ripens is an important biological factor in fruit distribution, and

the presence of the tannins in the unripe fruit keeps it from being eaten until the seeds are ready for dispersal.

5. According to Van Wisselingh, tannins play an important rôle in conjugation and cell wall formation in *Spirogyra*. Cells about to conjugate are always rich in tannins, which diminish as conjugation proceeds. During the formation of the cell walls after nuclear division, the tannin also diminishes. If the tannin was precipitated during the early stages of cell division, nuclear division continued normally but the cell wall was not formed. Such functions for tannin in higher plants have not been proposed.

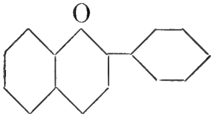
6. Tannins play an important rôle in cork formation, which explains their presence in bark. That such is the case may be concluded from three lines of evidence. (a) They occur abundantly in corky regions. (b) If a stream of carbon dioxide is passed through a mixture of formaldehyde and tannic acid, condensation products are formed which have many of the characteristics of cork. (c) Cork may be decomposed, yielding among other things gallic and tannic acid. The chain of evidence is thus seen to be quite complete. The formation of cork is important to the plant because it protects it against water loss and also against invasion by fungi after wounding. When trees are cut or pruned, a corky layer, which prevents infection, is soon formed over the cut surface.

7. The tannins may aid in some cases to maintain the turgor of the cell, the same as all substances in solution. Their presence in the vicinity of nectaries may be explained on this basis; and in the guard cells they may play a part in the opening and closing of the stomata. In evergreen leaves they may thus help to prevent undue evaporation in the winter and to retain the turgor of the cells in this time of physiological drought.

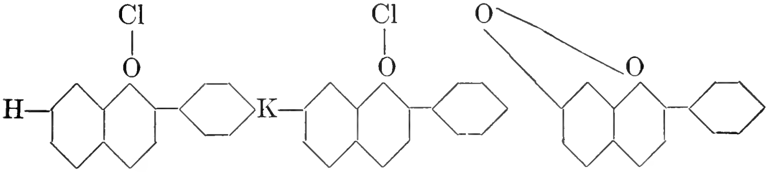
8. Finally, the tannins have an antiseptic value. Although fungi may use tannins as food in weak solutions, the concentration in the bark and leaves is generally high enough to retard or inhibit completely the growth of injurious fungi, especially parasites, which are more sensitive to tannins than saprophytes.

Pigments.—In addition to the chlorophyll and the carotinoids associated with it, other pigments are found in plants giving the characteristic colors of flowers, fruits, etc. These may be classified on the basis of their chemical structure as the anthocyans, anthoxanthins, and protein pigments.

The anthocyans are the soluble pigments in the cell sap to which are due the reds, blues, and violets of most fruits and flowers, of red cabbage, beets, red autumn leaves, etc. They are glucosides composed of a sugar and an *anthocyanidin* nucleus of the fundamental structure.



The anthocyans are ampholytes and react with bases and acids to form basic and acid salts, which vary in color from the bright reds, when acid, to the blues, when alkaline, according to Willstätter. The neutral anhydride salt is purple. Buxton and Darbishire (1927) found the sap of red flowers generally had a pH value of about 5.5 and that of blue flowers about 8.0. These different salts may be thus represented:



red blue purple

Intermediate colors may be produced by mixtures of different anthocyans or by mixtures of anthocyans with other pigments such as the anthoxanthins. Because of the change in color with acidity, the anthocyans are used as indicators in hydrogen ion and acidity studies in much the same way as litmus. When blue and red flowers die, they commonly change color owing to the changes in the acidity of the cell sap and protoplasm which accompany death. In *Ipomæa learii* the pink buds (pH 6.0) change to full blue (pH 7.8) as the flower opens in 30–40 minutes. As the blossom dies, the blue changes back through violet to pink.

The formation of the anthocyans depends primarily upon the presence of sugars. Other factors are more or less secondary. Light is important because of its importance in sugar formation, but the work of Shantz and of Arthur tends to show that this effect is indirect rather than direct; although the long rays are the most important in sugar formation, the short rays, notably the ultraviolet, are more essential to the immediate formation of the red pigment in flowers and fruits. Apples, peaches, etc., will not become red if kept in the dark, but the beet even when grown in the dark becomes red, possibly owing to the large amount of sugars present. In *Elodea* and other aquatic plants, Ewart (1897) found that the anthocyan would appear if the plants were grown in a weak sugar solution and strong sunlight. In water and diffuse light no pigment appeared. Low temperatures, which favor the storage of carbohydrates in the form of sugar, also promote anthocyan production, which may explain its presence in alpine and arctic plants. Similarly, unfolding leaves of hickory, oak, etc., are commonly red while much sugar is being sent into them from the winter storage tissues; and if the midrib of an *Œnothera* leaf is cut, the distal end becomes red as the sugars accumulate. E. Overton (1899), Boodle (1903), and Combes (1909) have all found that the amount of anthocyan present depends primarily upon the presence of soluble carbohydrates. In reddening cherries, the respiration quotient is such as to indicate that oxygen is being fixed inside the fruit, and Obaton (1923) suggested that this may be due to the fact that sugars are being combined with oxygen to form the pigments. But, in addition to the presence of requisite sugars, certain other inherited conditions, including the factors for the formation of necessary enzymes, must be met, as shown by Wheldale (1911). The sugar beet has even more sugar than the ordinary beet and yet it is not red.

The anthoxanthins also are glucoside derivatives of compounds like anthocyanidin called flavones and xanthones. The flavones are probably oxidation products of the anthocyanidins. To them are due the yellow pigments found in many plants such as yellow wood (*Morus*), osage orange (*Maclura*), and sumac (*Rhus*) which when extracted yield valuable dyes. They are not so common in yellow flowers and leaves, where the color is chiefly due to the carotinoids, but the pigments of *Antirrhinum* (snapdragon), sunflowers, and California poppy, are flavones as is that of yellow apples. The yellow coloring matter from onion skins, used in dying Easter eggs, is also a flavone derivative. Both the anthocyans and anthoxanthins are often associated with tannins, and it is possible that the tannins play some part in their formation.

The protein pigments or chromoproteins are found especially in the blue-green, brown, and red algæ, where they give the characteristic color to these groups. They are very complex compounds of high molecular weight (100,000–200,000) and seem to be compounds of pigments with proteins (presumably albumins). *Phycocyanin* is found chiefly in the blue-green algæ, while *phycoerythrin* is characteristic of the red. There are evidently two or three separate phycocyanins with separate shades of color and distinct absorption spectra. Phycocyanins have been reported also in some red algæ, while phycoerythrin has been reported in some of the blue-greens; but Danilov thinks these are related pigments and not genuine phycocyanin and phycoerythrin. Both these pigments are strongly fluorescent. The brown algæ have been said to contain a similar pigment, *phycophæin*, but some biochemists (Hooker and others) have doubted its existence and believe the color of these algæ is due entirely to the carotinoid pigment, fucoxanthin.

Physiological Significance of the Pigments.—Since pigments vary so much in different plants, they might be expected to have a variety of functions, if any. In considering the physiological importance of a material, it is often difficult to decide whether it really has a function or whether it may simply be the result of environmental (external or internal) changes and have no function, i. e., it is difficult to decide whether it has survival value or is merely neutral in its effect. What is it for? is the great question.

The pigments have been credited with these uses:

1. They aid in respiration. In the blood during animal respiration the hemoglobin is changed to oxyhemoglobin on the addition of oxygen in the lungs, with the reverse change in the tissues. The red pigment thus serves as an oxygen carrier, and Palladin (1909) has sought such a function in the plant pigments. The relation of carotin to xanthophyll, and the changes from red to blue anthocyans under the action of oxidizing and reducing enzymes, make such a function seem plausible. The relation of lycopersicin to the temperature has already been mentioned (Chap. V), and the same failure to become red occurs in the absence of oxygen. The connection between the anthocyans and the presence of sugars, which are easily oxidizable substances, also points to a relation between these materials and oxidizing phenomena (Chap. XXV).

2. They aid in photosynthesis by absorbing light, which may be used in the manufacture of food. Anthocyans absorb some of the rays which the chlorophyll does not and so are complementary to the chlorophyll. The relation of the algal pigments to photosynthesis has already been discussed (Chap. VI). Here the question

arises: Which come first, the anthocyans or the sugars? The facts favor the sugars.

3. They absorb heat rays. The red pigments certainly absorb some heat, and Keeble found that the side of a leaf where anthocyan was abundant was 2° C. higher than where no anthocyan was present. Ewart thinks this absorbing action protects the chlorophyll against too strong light, but the majority of workers seem to think that the purpose is to absorb as much energy as possible. This would be of advantage to alpine and arctic plants and to young developing shoots in the spring. The production of the red coloring matter in autumn leaves and in fruits permits more energy to be absorbed and thus hastens the metabolic processes of ripening which precede the fall of the leaf and fruit.

4. They attract or repel animals. Believers in such theories come to the rather paradoxical conclusion that animals are repelled by red foliage and roots but are attracted by red fruits. It is always dangerous to try to explain similar *appearances* by similar *causes.* In the case of fruits, the bright colors certainly have some survival value.

5. They result in cross-pollination in flowers. Most insect-pollinated flowers are brightly colored or have strong odors, and, while many investigators have shown that bees and other pollinating insects are very myopic, being guided much more by the odor than by sight, others have not been so easily convinced that the colors of flowers are unimportant. It seems hard to conceive that such conspicuous objects have not had some survival value in this connection.

6. They are important osmotic constituents of the cells. The same advantages may be derived in this respect from anthocyans and other soluble pigments as from any soluble compounds.

Autumnal Coloration.—The cause of the coloration of autumn leaves has already been mentioned. Upon the approach of cold weather the chlorophylls disintegrate and disappear. This permits the yellow carotinoids to be more easily seen and, at the same time, if sugars are present, the rich reds and lavenders of the anthyocans appear as they are formed. The browns are due to flavones or, more often, to tannins in the cell walls. Plants rich in sugars like the maples and birches are more likely to be bright red or yellow; while those rich in tannins, e. g., the oaks and beeches, are brown in color. Since the bright yellows and reds are dependent upon sugars, whatever makes for abundant sugar in the leaves makes for bright autumnal coloration. When the change from summer to winter is abrupt with early frosts, there will be more sugar in the leaves, since the leaves have been constantly at work with no diminution in their activity. Bright days produce sugars, and are also more likely to be followed by frosts than cloudy ones. Thus the conditions in regions like Eastern Canada and New England are ideal for brilliant autumn foliage. The frost comes early, the summer ends abruptly, and the autumn is bright, resulting in the gorgeous effects produced in these places.

Classification of pigments according to color and cause:

Brown—tannin, fucoxanthin, phycophæin (?)
Blue—alkaline anthocyans.
Green—chlorophyll, mixtures of yellow and blue.
Red—phycoerythrin, lycopersicin, acid anthocyans.
Yellow and orange—carotinoids, anthoxanthins, flavones.
Violet—neutral anthocyans.

QUESTIONS

1. What are terpenes? phenols?
2. Is phycoerythrin more nearly related to anthocyan or to lycopersicin?
3. What colors have flowers pollinated by night-flying insects?
4. Do flowers appear the same color to bees as to man?
5. Plants grown in the absence of nitrates are generally rich in anthocyan. Why?
6. Give the derivation of the following words: phycoerythrin, lycopersicin, phycophæin, anthoxanthin.
7. Name two ways in which tannins may protect against fungi.
8. What is an ampholyte? Give examples from this chapter.
9. Why is wine made from blue grapes generally red?

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CHAPTER XVI

ENZYMES

> Nor seed nor fruit have I,—
> Nor loss nor gain nor change in me is found.
> —JOHN TABB.

Catalyzers.—It has long been known by chemists that certain reactions between two substances would go on more rapidly in the presence of a third substance. Oxygen and hydrogen unite with explosive violence in the presence of spongy platinum. In a similar manner the breaking down of cane sugar, which takes place very slowly in a neutral aqueous solution, is accelerated greatly by the addition of a small amount of sulphuric or some other mineral acid.

Such accelerations have been called *catalytic* reactions, and the process, *catalysis*, has been defined by Ostwald as "the acceleration of a chemical change by the presence of some foreign substance." Such catalytic reactions are characterized in the following ways:

1. The catalyzer or catalytic agent (catalyst) does not appear in the end products of the reaction. It is not appreciably altered by the reaction and remains nearly the same in quantity and quality at the end of the reaction as at the beginning.

2. The catalyst does not initiate reactions but merely changes the rate at which the reaction would have continued without it. The reactions cited above are reactions which go on slowly in the absence of the catalytic agent but proceed at an increased rate in its presence.

3. The effect of the catalyzer is proportional to the amount present, if the amount of material acted upon is much in excess of the catalyzer, as is generally the case.

4. In proportion to the amount of catalyzer used, the effect of the reaction is very great. A very small amount of material is able to bring about a very great change; this is to be expected if the catalyzer can be used over and over again. Thus one gram of invertase may hydrolyze a million times its weight of cane sugar in a comparatively short time.

In all these four characteristics, catalyzers are seen to have the same effect upon the reactions that oil does upon machinery. *Theoretically*, the oil is not altered, it does not *start* anything, the effect is proportional to the amount used (within limits), and a small amount of oil may do a great deal of good. For these reasons catalyzers may be called the lubricants of chemical reactions.

Organic Catalyzers.—In test tubes, i. e., *in vitro*, the reactions which are produced by catalysis may often be produced by heating, which also speeds up the reactions. In organisms, however, the many processes which go on must be carried on at ordinary temperatures; otherwise the protoplasm is injured. The high temperatures produced *in vitro* cannot be produced *in vivo*. It would hence seem very advantageous to the organism to produce catalyzers which could bring about rapid reactions at ordinary temperatures. Such catalyzers have been found in great quantities and of many kinds, so that every conceivable kind of reaction known to be necessary in the organism may have its own catalyzers. To them have been given the name of *enzymes*, which may hence be defined as *organic catalyzers*. The name means "in yeast" (Gr., *en zymos*) and has been chosen because the fermentations produced by yeast were among the first of such reactions to be carefully studied.

Organized and Unorganized Enzymes.—It was first thought by Pasteur and the workers of his period that the fermentation was a direct result of the action of the *living* yeast and that it was a property peculiar to the yeast cells. Later it was found that water extracts from sprouted barley seeds, from the stomach, and from other tissues where sugars were broken down, had the same property. Such extracts were called *unorganized* ferments as opposed to the *organized* ones represented by the living yeast cells. When Büchner, however, in 1897 showed that, by grinding the yeast cells with sand and expressing the liquid under pressure, he could obtain a liquid free from all living cells and which was just as active as the yeast plant itself, all differences between organized and unorganized ferments disappeared. Instead one finds the terms intracellular and extracellular enzymes. The former are not secreted out of the cell, but the cell must be crushed in order to secure the enzyme. These correspond to the "organized ferments." The extracellular enzymes, on the contrary, are secreted by the cell into the liquid or into the intercellular spaces outside of the cell. Here, as in the intestine, the pitcher of the pitcher plant, etc., they do their work. The relation to the cell is secondary as long as the proper environment (temperature, acidity, etc.) is maintained.

General Characters.—Enzymes have all the characteristics named above for inorganic catalysts and, in addition, possess others which may be enumerated as follows:

1. They are colloidal, which means that they possess the general properties of such materials as outlined in Chapter II. They carry electrical charges, exhibit to a marked degree the phenomenon of *adsorption,* and do not diffuse through parchment membranes. They do, however, pass through porcelain filters, and this fact is made use of in separating enzymes from the cells which contain or secrete them.

2. They were once thought to be proteinaceous in nature, but it is extremely difficult to secure the enzyme in a pure condition, and it is possible that the protein material is an impurity. Although urease, the first enzyme to be isolated (by Sumner, 1926), was found to be a protein of the class of globulins, the more that enzymes have been purified, in general, the fewer protein characteristics do they show; and Willstätter has shown definitely that lipase, invertase, and peroxidase are not proteins. Peroxidase, however, is spoken of as a "nitrogenous glucoside." Some enzymes retain their activity when acted upon by proteolytic substances which would destroy them if they were proteins. This fact would indicate that the protein is an impurity, and that the colloidal inorganic salts such as those of manganese and iron which are often found associated with enzymes, may be the important constituent. It may develop that their *chemical* nature is not so important, and that their *colloidal* nature is the chief factor in their action. It may also develop that some are proteins and others are not.

3. They are very sensitive to heat and light. Each enzyme has an optimum temperature which is generally between 40° and 55° C. Most of them cannot be heated over 70°, and all are inactivated or "killed" at the temperature of boiling water (100° C.) if in solution, although, when dry, they may be able to stand slightly higher temperatures for a short time. The inactivation by heat may be explained most easily as a coagulation of the colloidal proteins present. The activity of the enzyme is doubled

for every rise of 10° C. according to Van't Hoff's general statement for chemical reactions, but the deleterious effect of the heat upon the enzyme at the higher temperatures tends to diminish the effect after the optimum temperature is reached. At that point, the harmful effect upon the enzyme is greater than the beneficial effect upon the reaction.

Light also favors the destruction of the enzyme. Ultra-violet light and the shorter wave lengths are especially deleterious, while the harmful effect of ordinary sunlight is much increased by the presence of oxygen and depends largely upon it.

4. They are very sensitive to acids, alkalies, salts, etc. Each enzyme works best in a medium of definite pH value and any change from this optimum is seen in the action of the enzyme. Thus pepsin works best in the acid medium of the stomach, while trypsin, in the intestine, requires an alkaline medium. A very small amount of excess acid or alkali inactivates an enzyme. This also may be explained on the basis of the colloidal nature of the enzymes. Colloids, as has been noted, are especially sensitive to carriers of electrical charges such as acids, bases, and salts. Enzymes, however, are less affected by antiseptics, such as toluene, which injure protoplasm.

5. With few exceptions they are very specific in their action. Only a limited number of substances can be acted upon by any given enzyme, and in many cases only one. Thus invertase acts only upon cane sugar and lactase upon milk sugar; but maltase is able to hydrolyze both maltose and alpha methyl glucoside; and emulsin, one of the glucosidases, can decompose milk sugar, amygdalin, arbutin, salicin, coniferin, and still other substances. It is probable, however, that emulsin is really a mixture of enzymes.

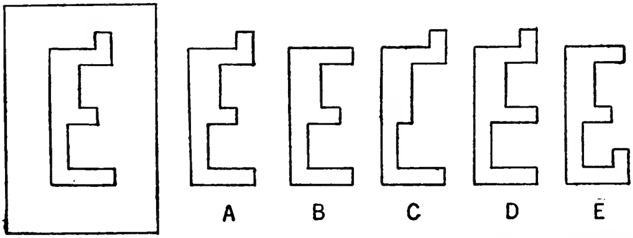

FIG. 8.—Illustrating the lock and key hypothesis of enzyme specificity. A key the shape of A, B, or C would fit the lock and might unlock it, while a key the shape of D or E could not be made to enter the lock.

This specific action may be explained on the basis of the lock and key hypothesis, which was first proposed by Pasteur to explain why an enzyme would attack a dextro sugar and not a levo one. If we assume that the compound contains some special grouping or configuration of molecules which the enzyme may "fit," then only enzymes with the corresponding configuration will be able to "unlock" the compound and decompose it (Fig. 8).

Classification of Enzymes.—Enzymes are classified according to the work they do and are named with endings in *ase.* They are thus classified as follows:

I. Hydrolases.
  a. esterases.
  b. carbohydrases.
  c. glucosidases.
  d. proteases.
  e. urease.

II. Oxidases.
  a. oxidases proper.
  b. peroxidases.
  c. catalases.
  d. reductases.

III. Fermentases.

IV. Carboxylases.

V. Coagulases.

The hydrolases are concerned in the reactions of hydrolysis, which accompany digestion, and will be discussed in Chapter XXII. The oxidases and fermentases are concerned primarily with respiration and the release of energy. They will be discussed more fully in Chapters XXIV and XXV.

The carboxylases split organic acids into carbon dioxide and the remaining aldehyde, ketone, or amino group. Thus pyruvic acid is split up into carbon dioxide and acetaldehyde, according to the equation:

$$CH_3CO.COOH \rightarrow CH_3COH + CO_2.$$

The coagulases, which bring about the coagulation of various classes of materials, have been mentioned before. Rennin is the enzyme of milk which causes coagulation or curdling and is of much importance in cheese-making. Pectase, which coagulates soluble pectin bodies to a jellylike mass in the presence of calcium, is in this group of enzymes. Vitellase (Lagrange, 1926) from bacteria and molds permits them to coagulate egg yolk and, therefore, belongs in this category.

Variety in Plants.—The number of enzymes which a plant may contain is surprisingly large. From the leaves of the common beet have been taken invertase, diastase, and maltase; from the stem, invertase, diastase, inulase, and emulsin; and from the root, all except the invertase. In the secretions of pollen tubes have been found amylase, invertase, catalase, reductase, pectinase, proteases, lipase, and zymase. Between fifteen and twenty enzymes have been isolated from molds, which can produce different enzymes according to the kind of food they are using. Other observers find, however, that many enzymes are produced regardless of the nature of the substrate.

Many enzymes may exist in the plant which have not yet been found, and probably all or most reactions in the organism will ultimately be found to be catalyzed by the action of enzymes. These processes are generally spoken of as "controlled by the action of the living protoplasm," but this phrase explains nothing, and all such reactions are probably catalytic.

Analysis and Synthesis.—It was formerly thought that enzymes caused only decompositions, and the syntheses were explained by the "action of the living protoplasm" just mentioned. There is now ample evidence that the enzymes cause both synthesis and analysis depending upon the environment,—acidity, presence of the reacting substances, etc. The surrounding conditions determine, for example, whether dextrose is to be built up into maltose and starch or whether starch will be torn down into maltose and dextrose. The same enzyme can cause the reaction to proceed in either direction, depending upon the conditions in the cell where the work is taking place. This idea, that the enzyme which accelerates the decomposition may accelerate the synthesis, has been proved in the case of maltase, which has been used for synthesizing maltose from glucose. In a similar manner artificial fats have been synthesized by the aid of lipase from the pancreas, simple proteins from amino acids by the aid of proteases, and glucosides from their components by glucosidases.

Mode of Action.—Much discussion has been held over the action of the enzymes. How do they bring about the acceleration of the reactions? The prevalent theories may be classified as follows:

1. Mere adsorption—*physical.*
2. Some kind of *chemical* combination.
   a. colloidal.
   b. molecular.

Those who hold to the adsorption theory consider that the enzyme draws the two reacting substances so close together that they react with each other. The glucose molecules, for example, are adsorbed so tightly by the molecules of maltase that two of them unite to form maltose. This attraction may be of an electrical nature or be due to some as yet unknown cause.

Those who believe in a chemical combination theory hold that the enzyme unites in some way with the reacting substances and thus brings them together. When its work is done it withdraws and then unites two others. The former (adsorption) theory may be compared in its action with that of the village matchmaker who brings the two parties together in her parlor and thus increases the chances of their union, while the action demanded by the latter theory is more comparable to that of the parson who takes the hand of each and places it in that of the other, after which he withdraws and goes on to the next couple.

The nature of this chemical combination is not well understood. Is it a sort of colloidal reaction or is it a chemical one in the ordinary sense where molecules and atoms enter into new combinations? The effect of heat upon the reacting substances; the appearance of the materials under the ultramicroscope; the fact that if an enzyme and its substrate, each of which will pass through a given filter, are mixed, the reacting mixture will not pass through the filter; and the general colloidal nature of the enzymes, etc., all point to a colloidal combination (e. g., adsorption), and the weight of the evidence is in favor of this viewpoint; but the specific nature of the enzymes might be considered as a strong argument in favor of some kind of molecular combination.

Willstätter's conception of enzymes is a sort of compromise between these two views. He thinks that the enzyme consists of a colloidal complex or carrier, to which is attached the specific catalytic group. This latter group possesses particular affinities for definite molecular groupings of the substrate and explains the chemical properties of the enzyme and its specificity. Thus, if we assume that melezitose (Chap. XII) is glucose-levulose-glucose, while raffinose is galactose-glucose-levulose, an explanation is at hand as to why *Aspergillus* invertase can hydrolyze melezitose but not raffinose, while yeast invertase acts *vice versa.* In the first case, the invertase attaches at the glucose molecule and in the second case on the levulose. The colloidal carrier explains the colloidal properties of the enzyme; when the properties of this aggregate are destroyed (e. g., by heat or salts), the activity of the enzyme is destroyed. This is a genuine masterpiece of hypothesizing, but, until more enzymes are isolated, these points, like many others in plant physiology, will remain unsettled.

Accelerators.—The activity of enzymes is much influenced by the addition of small quantities of an electrolyte (acid, alkalies, or salts) as previously mentioned. This action when favorable is said to be *accelerating.* It may be connected with the electrical charge of the colloidal enzymes and their general properties as colloids; but some ions have a very specific effect, as shown by Doby and Hibbard (1927), who report that amylase is strongly accelerated by Cl^- ions but only weakly by K^+ and NO_3^-. Saccharase, on the other hand, is strongly accelerated by NO_3^-, less by K^+, and is paralyzed by Cl^-. A different type of accelerating action, which is probably not electrolytic, is that of certain amino acids such as asparagine upon amylase.

Inhibitors or Paralyzers.—Inhibitors destroy or inhibit the action of enzymes. The electrolytes mentioned above are inhibitors when harmful. Many other substances exercise an inhibitive or toxic action, e. g., mercuric chloride, hydrogen sulphide, formaldehyde, hydrocyanic acid, etc. In fact much of the harmful effect of these materials on the general organism may be traced to their effect upon the enzymes. Of the heavy metals, mercury, silver, and copper are the most toxic.

Coenzymes.—In many cases the enzyme is accompanied by some substance, the presence of which is absolutely necessary for its action. This accompanying material may be separated from the enzyme by dialysis and then boiled without losing its power. When added to the enzyme the activity is resumed. This may be repeated any number of times. Such accompanying substances are called coenzymes. The separation of the bile salts from liver lipase is an illustration from the animal world of the separation of a coenzyme from its enzyme; while the relation of phosphates to yeast zymase (Chap. XXV) and of calcium salts to pectase are similar ones in the plant kingdom.

Activators or Kinases.—In some cases the enzyme is powerless when it originates and must come in contact with another substance which renders it active. The enzyme in this case is not considered a real enzyme but a *proenzyme* or *zymogen,* while the substance which renders it active is called the *activator* or *kinase.* This kind of action should not be confused either with the acceleration produced by acids, etc., which act upon enzymes already active, or with that produced by coenzymes. A coenzyme may be separated from its enzyme and the action is reversible, which is not the case with an activator and its zymogen. Trypsinogen, secreted by the pancreas, has little action on proteins until it is activated either by calcium salts or by the enterokinase secreted by the intestine.

Proenzymes have likewise been reported from plant tissues. They are said to be present in the cells as small granules which disappear when the zymogen has been activated. Thus prolipase has been reported from castor beans, proöxidase from tobacco leaves, and propapain from *Carica* (activated by HCN). The fact that dilute acids and calcium salts are excellent activators of zymogens has led Waldschmidt-Leitz to deny the existence of such proenzymes. His position (and a not unreasonable one) is that these zymogens are simply inactive because of improper pH or some similar factor. The "activators" do nothing to the enzyme, but merely change its environment to a more favorable one.

Antienzymes.—An antienzyme is a substance occurring in the living organism (or produced in it by the injection of an enzyme) which prevents the action of an enzyme upon the tissue. Thus intestinal worms and other parasites of the digestive tract must secrete some antipepsin and antitrypsin as well as other antienzymes to prevent their digestion by the digestive juices in their environment. Similar enzymes probably exist in the lining of the alimentary canal itself to prevent autodigestion. Since mosquito larvæ can hatch and develop in the pitchers of pitcher plants, the larvæ probably contain antiproteases. In plants, antioxidases have been reported by Czapek (1905), and it is reasonable to expect that the protoplast must always contain antienzymes to protect itself against the injurious action of the enzymes it secretes. Whether these antienzymes are a specific group of compounds, however, is doubtful.

Summary; Artificial Enzymes.—The reactions of plants at life temperatures are hastened by organic catalyzers called enzymes. These are probably proteinaceous in nature but owe their properties more to the fact that they are colloids than to the fact that they are proteins. This is borne out by the experiments of Bredig, who prepared colloidal catalysts from silver and other metals by passing 110 volts of electricity between metal electrodes in a water solution, thus preparing a solution of the colloidal metal which had the following characteristics of enzymes:

1. They had an optimum temperature.
2. They had an optimum acidity.
3. They were poisoned or inhibited by hydrocyanic acid and other toxins.
4. They acted both synthetically and analytically.
5. They were colloidal.

QUESTIONS

1. Why does sweet corn lose its sweetness unless cooked soon after its removal from the plant?
2. What is the source of the production of enzymes?
3. What is fermentation?
4. Distinguish between catalase and catalyze.
5. Compare the effect of heat on dry and moist enzymes.
6. Distinguish between an inhibitor and an antienzyme.

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CHAPTER XVII

HORMONES, VITAMINS, AUXIMONES

> Man shall not live by bread alone.
> —MATTHEW 4:6.

Within the past two decades, many experiments have been made which show that in addition to the foods and allied materials mentioned in the previous chapters, plants and animals need still other materials in order to develop normally and completely. These materials seem in some cases to be taken into the body along with the food and in others to be manufactured inside the body. Here, as elsewhere, plants are more independent and self-supporting than animals. They are able to elaborate or make most of these materials by the activity of their own protoplasm, while animals are dependent upon plants for many (but not all) of these substances.

These materials not only have a helpful or beneficial effect upon the physiological processes of the plant which produces them, but also upon the animals which get these substances from the plants. Depending upon their function, their method of operation, and the way in which they are produced, these accessory factors have been variously treated by different workers under the names of hormones, vitamins, and auximones. Whether these are all distinct substances remains to be settled, but the general nature of the materials will be briefly discussed.

Hormones.—Vöchting (1878) noticed that if willow shoots are cut and hung up in a moist place, roots will appear at the lower end and shoots at the upper end. If some of the pieces are now inverted so that the end formerly towards the root is uppermost, roots will develop only at the upper end, where they are useless, and stems only at the lower end. It thus appears that the one end is a "root end" and the other a "stem end," and that roots and stems develop at these places regardless of their utility or of such external conditions as gravitation and light. Furthermore, if a section of such a stem be broken up into pieces, each piece will have a root and a stem end, the root end being the one nearest the root as it grew and the stem end the one nearest the end of the stem. Sachs (1882) explained this on the basis of stem- and root-forming substances. Materials were formed in the plant, according to this hypothesis, which determined the formation of roots and migrated toward them, so that there was always more of this "root-former" in the root end of a piece than in the stem end. The stem-forming materials formed a similar gradient in the reverse direction, with the result that one end was a *root* and the other a *branch* end. He said: "Just as organs differ in form, so there is a corresponding diversity in the materials which compose them. These organ-forming substances act like ferments upon large masses of plastic material although they themselves are present in very small amounts." We now use the term ferment in a very different sense (Chap. XVI), and for the material which Sachs had in mind, Starling (1905) proposed the word *hormone.*

Hormones, or chemical messengers, are compounds which are produced in one part of the organism and, when carried to other parts, there bring about very marked and striking changes. The word was first used in animal physiology where much more work on hormones has been done. Here they go under the common name of "internal secretions" and include the products of the ductless glands such as the thyroid, adrenal, pituitary body, and the internal secretions of the gonads. These chemical compounds when carried to the other parts of the body result in normal growth and in the normal development of the secondary male or female characters, or else in gigantism, homunculism, a goitrous condition, etc., depending upon whether they are present in normal or abnormal amounts. As Brown-Sequard has shown, these secretions may influence not only the entire physical character of the body but also the psychic life and the mentality of the organism.

It was previously thought that the chief connection of the parts of the body with each other was through the nervous system, but now we know there are these chemical connections as well. As Biedl (1910) sums up the matter: "The theory of internal secretions now plays an important rôle in nearly every problem of physiology and pathology and is very important in general biological problems. Schiefferdecker's hypothesis concerning the part played by specific internal secretions in the control of the nervous system shows better than anything else the recent change in our attitude toward the rôle of these secretions. . . . These concep-

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Dr. F. E. Denny, Plant Physiologist at the Boyce Thompson Institute, Yonkers, N. Y. *Plant biochemistry; Dormancy; Effects of stimulating agents.*

tions of nervous control are now generally accepted but they clearly show how our attitude toward nervous activity has changed in the past few years."

Hormones have not been isolated from plants or demonstrated so clearly as in animals, but their presence is inferred to explain the physiological changes mentioned and described below, which are otherwise inexplicable.

Thus Errera (1904) explained by means of internal secretions the changes in geotropism observed in spruce branches. The leader or central axis of a spruce is negatively geotropic while the lateral branches are diageotropic; but if the leader is removed, one of the lateral branches will then become negatively geotropic and grow up as though trying to replace the lost tip and carry on the functions of the leader. According to Errera, the apical shoot secretes an inhibiting substance which keeps the other branches diageotropic in their normal position, but when the main tip is removed, the source of the inhibiting hormone is likewise removed and a lateral shoot then assumes the functions and characteristics of the lost leader.

Bruck (1904) observed the same response in roots. When the terminal two millimeters of the primary root is removed, the lateral roots just above become positively geotropic and bend downward to replace the removed organ.

Vöchting (1908) showed that in rape (*Brassica rapa*) when the blossoms were removed the uppermost leaf became vertical instead of lateral as normally. Similarly Bässler (1909) showed that in many annuals which bear no branches, the cutting off of the stem results within twenty-four hours in an upward bending of the leaves nearest the cut of from five to thirty degrees or even more. Those nearest the cut bend up the most. All these examples can be explained in the same manner; and further support is given the hormone hypothesis by the fact that a *vertical* cut, which would not interfere with the transport of the hormone, does not cause the response by the lateral leaves or branches.

Similarly Loeb (1915–1924) concluded that the development of buds and roots on the leaves of the live-forever (*Bryophyllum calycinum*) was prohibited by a hormone secreted by the apical bud, since the buds lower down on the edge of the leaf will not develop until the apical bud is removed. The removal of this apical bud shuts off the flow of hormones from it. Since the bud nearest it is the first to be freed from the inhibiting effects of the hormone, it is the first to grow out, producing new hormones (i. e., inhibitors), which then check the development of the other buds. The degree of inhibition depends upon the size of the bud and the amount of hormone produced, so that under certain circumstances the inhibition extends only to those primordia near the apex.

On the basis of the hormone hypothesis Swingle (1926) has explained the fact that date pollen influences not only the character of the date seed, which may be explained by xenia, but also influences the size, shape, character, and time of ripening of the fruit. He believes that the embryo or endosperm of the date seed, or both together, constitute a ductless gland apparatus which by secreting hormones affects the development of the entire date fruit. Montemartini (1929) found that when a red-hot wire was thrust into an unripe pear, penetrating and killing the seed, the ripening of the pulp was greatly accelerated. This also is interpreted as the effect of a hormone which diffuses out from the seed into the fruit.

Likewise Went (1926) has explained the phototropic response of oat seedlings as due to a photoreactive hormone which is produced at the tip of the coleoptile and descends on all sides. Its function is to increase the elasticity of the cell walls and permit the cell to increase in size (grow). Light checks the descent of the hormone, so that when illuminated even an extremely small amount, the hormone passes down only on the shaded side, where growth then occurs, with the result that the plant bends towards the light. The hormone is thus seen to be a "growth hormone" rather than a "tropohormone." These results have been confirmed by Söding (1925–1929), who also found that when decapitated coleoptiles were reattached by a gelatin bridge, growth was more rapid than when the tips were not replaced. When peduncles of *Cardamine* and *Cephalaria* were cut off and reattached by agar, similar results were observed, which indicate that there is some substance, i. e., a hormone, which can cross the agar or gelatin bridge and affect the growth of the parts below.

Boysen-Jensen and Paal have reported like results, and Snow found that the geotropic stimulus in *Vicia faba* can be transmitted across protoplasmic discontinuities. Perry (1932) found that the coleoptiles of grasses in general and of oats in particular contain cells which seem to be secretory, thus giving a histological basis to the hypothesis of Went.

Haberlandt interprets the growth of cells near a wound as due to a wound hormone that diffuses out from the injured cells. If the surfaces are washed after cutting or wounding, fewer meristem cells form than when pulp of crushed cells is spread over the surface.

It must be remembered that some writers (Child and others) consider that, since hormones have not been isolated, these phenomena in plants can be explained best by the conduction of stimuli rather than by the transference of definite, tangible, chemical compounds. Furthermore, cooling a petiole to 3° C. results in physiological isolation, which points to a *stimulus* rather than a *hormone*, according to Child; but the transfer of materials in plants is also checked by a decrease in temperature and so this argument does not seem to be extremely weighty. Since only small quantities are necessary and since hormones are known to exist in animals, it seems only reasonable to assume their existence here in light of the phenomena described. True, there is not the means for rapid distribution in the plant as in the animal, but there is the means for a slow distribution and the responses in plants are in general not so rapid as in animals. The transfer of a stimulus should take place much more rapidly than the responses cited above. Thus the time factor would favor the hormonic rather than the "nervous" theory in the cases cited, even though, as will be stated later (Chap. XXVIII), there are cases in plants where the response is thought to be due to what may be called an elementary nervous stimulus. As to the criticism that hormones have not been isolated in plants, it is claimed by some authors that many of the more common substances such as the glucosides and alkaloids have an hormonic effect in stimulating growth and development.

The hormones are thought to stimulate the action of enzymes, which in turn bring about the metabolic changes we find in plants. The hormone is thus the "trigger" which excites enzyme activity and causes the enzymes to be secreted. Similar results in the formation of galls and other excrescences are produced by the injection of materials when the plant is stung by gall insects, and thyroid extract has been found to promote enormously the growth of hyacinth bulbs and potato tubers. How the hormones bring about these changes is still unknown, but Denny (1926) found that the dominance of the apical bud in potato tubers can be checked by thiourea; in place of only one, two to five buds then develop from each eye. It has been suggested that hormones change the permeability of the protoplasm thus causing changes in the osmotic conditions inside the cell. Others have proposed that they cause a separating out of the constituents of the protoplasm, much as freezing might, and in this way aid in secretory activity. The complete chain of chemical reactions still remains to be worked out; only a few links have thus far been forged.

Vitamins.—Of all the substances in this category which have attracted the attention of the public and of physiologists alike, the vitamins are most conspicuous. This is because they have been found to play such an important part in animal nutrition. Funk (1911), Osborne and Mendel (1913–1915), as well as many other investigators including Sir Frederick Hopkins, have shown that animals cannot live and thrive on pure foods. In addition to the foods and ordinary mineral salts which animals must have, there must also be present for normal growth and development certain "impurities," to which Funk has given the name *vitamins*. These vitamins are not foods but food *accessories*, which have specific stimulating or regulating effects and thus determine the normal physiological operation of the organism. Comparatively little is yet known about their chemical nature, with the exception of vitamin D, but this little is sufficient to tell us that the name, which implies the presence of an amine group, was unfortunately chosen since the majority of vitamins contains no such groups.

At the present time six of these vitamins have been especially studied and inasmuch as two of these have been definitely added to the list during the past five years, it is probable that all have not yet been found. These vitamins, called A, B, C, D, E, and G are made for the most part only by plants, with the result that animals are dependent upon plants for them. They (animals) do not have the power to make their vitamins from foods which lack them. Fish oil, for example, which is rich in certain vitamins, has the same odor as the oil of diatoms, which play a large part in the diet of fish. The absence of any one of the vitamins causes a certain characteristic disease known as a deficiency disease (avitaminosis), which with the corresponding vitamins will now be briefly described.

Vitamin A.—This vitamin is found in dairy products, fats and oils, egg yolk, carrots, leaf vegetables in general and spinach especially. Liver oils and dairy products are very rich in this vitamin, which is fat soluble. Owing to the fact that it seems to be associated with the carotinoids in leaves rich in chlorophyll, in carrots, tomatoes, yellow corn, egg yolk, etc., it has been proposed that it might be identical with one of the carotinoids. In plants or parts of plants which contain no carotinoids, this vitamin has never been found, and Mangelsdorf and Fraps (1931) found that the amount of vitamin A in yellow corn varied with the number of genes for yellowness present. Furthermore, white-seeded varieties with little or none of this vitamin can form it when the gene for yellow color is introduced by pollinating with a yellow-grained variety. If vitamin A is not carotin, it is suggested by Capper that it may be formed from carotin in the animal body, since rats suffering from A-avitaminosis recovered when given carotin. Hence, although it has not been definitely proved that vitamin A is a carotinoid, their simultaneous occurrence in the same places must be of some significance, and it is probable that carotin is a precursor of A, i. e., its provitamin.

The absence of vitamin A from the diet causes growth to stop and produces an opacity of the cornea of the eye called xerophthalmia, which may result in total blindness but is speedily relieved by the addition of a little spinach and cream to the diet. Its absence from the diet also causes a general weakening of the tissues and a degeneration of the mucous membranes, which renders them especially susceptible to infections. Night blindness (hemeralopia) seems also to be caused by the absence of this vitamin from the diet.

Vitamin B.—This vitamin is water soluble and is found in fresh vegetables, nuts, fruits, and yeast. The grains of the cereals contain it in abundance, especially in the outer coat. It is not found in sugars, starches, or fats, with the result that diets rich in these foods will be short in vitamin B. Its absence in the diet of animals causes polyneuritis and, in man, the diseases beriberi and neuritis. For this reason, vitamin B is commonly called the "antineuritic vitamin." Beriberi, which is associated with œdema and a weakened heart, is found among rice-eating peoples if the rice has been polished, in which case the pericarp containing the vitamin is removed. If the entire grain is eaten, immunity from the disease is insured. It is interesting to note that of all the vitamins, this and vitamin G are the only ones found in plants lacking chlorophyll. Yeast is one of the best sources of this vitamin and is the one generally used to cause recovery from experimental B-avitaminosis. Vitamin B can apparently be synthesized by yeast as well as by other microörganisms, including various molds and bacteria (Sunderland and Werkman, 1928).

Bios.—In 1901 Wildiers noticed that yeast did not grow well on a purely synthetic medium, and explained this by assuming that the yeast required some unknown substance, to which he gave the name of *bios*. He believed that the yeast could not produce its own bios but must get it from external sources. The word "bios" has since been reserved for growth-promoting factors for yeast, but the term is still very much confused and is used by different workers to mean (a) a growth stimulant, (b) an essential factor for growth, and (c) a factor stimulating the production of carbon dioxide. Some kinds of yeast seem to require it for growth, while for others it acts only as a stimulant.

Also much has been written to prove and to disprove that bios is the same as vitamin B. The evidence against the identity of these two substances is now overwhelming, but they are, nevertheless, distinctly related. While some yeasts will grow without bios in the medium, they do not have any antineuritic potency, which has led Funk and others to the conclusion that the yeast requires bios for the synthesis of vitamin B. This would indicate that perhaps bios is a sort of provitamin. The word "nutrilite" has been proposed by Williams (1928) to include such vitaminlike substances.

Vitamin C.—Vitamin C is also water soluble and is found in fresh vegetables, milk, and fruits, especially in citrus fruits and tomatoes. It is very unstable and seems to be more sensitive to heat than the other vitamins, although cooking will not entirely destroy any of the vitamins if they are kept in a closed vessel when heating, which implies that the chief injury is through oxidation rather than direct destruction by heat. Children, however, who are fed on pasteurized milk, should be given a supplementary diet of fruit juices.

Vitamin C is called the antiscorbutic vitamin since its absence results in the disease known as scurvy. Formerly sailors and explorers who were deprived of fresh fruit and milk were especially subject to this disease, the symptoms of which were hemorrhages, soft gums, and "rheumatism of the legs." A small amount of orange juice is sufficient to prevent the disease. It is interesting to note in this connection that dried peas and beans do not contain this vitamin, while the same seeds when sprouted are very efficient antiscorbutics. Matsuoka (1930) also reports that rice, barley, and oats have much more vitamin C when sprouted than when unsprouted.

Vitamin D.—This is a fat soluble vitamin and for some years was confused with A. It is found in the same general regions as A but liver oils are especially rich in it and codliver oil contains it in very large amounts. Of all the vitamins, this is the one found least in plants, which are almost entirely devoid of it. However they do contain the parent substance, ergosterol, which, when irradiated with ultra-violet light, becomes vitamin D. This vitamin controls the calcium and phosphorus metabolism of the body and its absence results in the disease known as rickets, in which the bones are weak and fail to develop properly. During the World War, the children of Germany and Austria suffered especially from this malady owing to the shortage of fats in these countries and the fact that children are always the first to feel the effects of a deficient diet. It has been learned that the ultra-violet light of sunlight is also a cure for rickets and that animals can synthesize this vitamin from ergosterol in the presence of sunlight containing these short rays. Likewise plants containing ergosterol, i. e., provitamin D, can synthesize the vitamin when irradiated, as stated above. Thus spinach leaves, which are only slightly antirachitic, become powerfully so when irradiated with ultra-violet light. Steenbock and Black (1924) showed that even synthetic diets deficient in vitamin D can be endowed with antirachitic powers when exposed to ultra-violet light, if they contain ergosterol. Thus margarine may be given the vitamin D properties of butter by irradiating it with ultra-violet light, and codliver oil is truly "bottled sunlight."

Vitamin E.—Vitamin E is also fat soluble and found in the seed germs of cereals, lettuce leaves, and wheat oil in particular as well as in other cereals, green vegetables, and seeds. In its absence, the placenta of the female breaks down so that reproduction is impossible, but since the gonads are not seriously affected, this sterility can be cured. In the male, the testes degenerate, resulting in permanent sterility. For this reason vitamin E is spoken of as the reproductive vitamin.

Vitamin F.—This vitamin, if one may predict in such matters, is to be one of the vitamins into which B is at the present time being split. The name seems to be settling on a growth-promoting factor found in leaf vegetables and liver, but at the moment the research in this field is too confused to be discussed in an elementary, general text such as this.

Vitamin G.—After researches and disputes lasting over many years, it has been concluded that pellagra is a deficiency disease due to the absence of this vitamin. This disease occurs in the southern United States, where corn and fat pork (with very few vegetables) are the staple articles of diet. The symptoms are much like those of beriberi, and it is, therefore, not surprising that this vitamin was for a long time confused with vitamin B. It is water soluble, and its chief sources are yeast, lean meat (especially glandular organs), green vegetables dairy products, eggs, fruits, and fish.

The following table shows the general distribution of the vitamins as determined up to the present time (the figures indicate relative richness):

Food	*Vit. A*	*Vit. B*	*Vit. C*	*Vit. D*	*Vit. E*	*Vit. G*
Cereals	1	2	–	–	3	1
Dairy products	2	1	1	1	1	2
Eggs	2	2	–	2	2	2
Fruits	1	1	3	–	1	1
Leguminous seeds	1	2	2	1	2	1
Meats	1	1	1	1	2	3
Nuts	1	2	1	–	1	1
Oils and fats	3	–	–	2	1	–
Vegetables	2	2	3	1	3	2
Yeast	–	3	–	–	–	3

Vitamins and Plants.—While it has been known that, with the exception of vitamin D, vitamins are manufactured only by plants, they have been studied chiefly from the point of view of their effect upon animal nutrition. Whether they are equally necessary for the nutrition of the plants which produce them, remains to be shown, but the fact that the plant organs such as fruits, leaves, and seeds, where metabolic processes are very active, are the organs richest in vitamins, would indicate that they do play a part in the economy of the plant. Although Lumière (1921) concluded that vitamins were not necessary for plants, from the conclusions of most investigators it would appear that vitamins *are* as essential to plants as to animals; but the green plant has the power to make its own vitamins in the same way that it can make its own food.

Since green plants can make their own vitamins, it is impossible to grow them in a vitamin free medium, as can be done with animals, and this makes the effects of vitamins upon plant growth very much more difficult to study. But, from the results fairly well established, it seems probable that saprophytes and parasites, which do not make their own food, are like animals in that they cannot make their own vitamins; they are dependent upon the presence of these materials in the nutrient medium. Their food must contain some vitamins of plant origin in addition to the sugars, mineral salts, etc. This has been shown to be true for the brown rot fungus of peaches and plums (*Sclerotinia cinerea*), for yeast, and for many kinds of bacteria. The yeast, which itself is an important source of vitamins, obtains them from the nutrient medium upon which it grows, and it is probably for this reason that barley malt is so stimulating to the growth of this fungus. But this leads us back to the relation between vitamin B and bios, where we have seen the difficulty in determining the relation between vitamins and similar substances and have noted some of the problems that arise in trying to determine what organic materials a plant takes in from the surrounding medium and what ones it manufactures within its own body.

Auximones.—Bottomley (1917–1920) concluded that plants required something more than the elements generally admitted as necessary for plant growth and found in ordinary culture solutions. These growth promoters, of which the nature and composition were unknown, he called *auximones.* For his experiments he used water plants such as *Lemna* and *Azolla* in order that the ordinary difficulties involved in water cultures might be eliminated; and he found that for good healthy growth, small amounts of organic materials were necessary in the culture solution. The best source of this organic matter is bacterized peat, which is peat produced from *Sphagnum* (a moss) that has been acted upon and decomposed by nitrogen bacteria. This bacterized peat acts as a food accessory rather than as a food, as shown by the very small quantities required. For this reason it has been thought to be a vitamin and has been so called by some workers. Rosenheim

(1917), for example, found that the primrose, *Primula malacoides*, when treated with an aqueous extract of only 0.18 g. of bacterized peat, grew noticeably taller than plants not so treated. This small quantity of peat contained only 0.02 g. of organic matter, of which only about one-tenth or 0.002 g. was nitrogen, so it is hard to understand to just what is due the stimulating action. Likewise Ashby found that while *Lemna* lived indefinitely in a purely inorganic solution, one part in five million of an organic extract from horse dung was sufficient to significantly increase the growth rate. He attributes this action to that of a catalyst. Breazeale also found in manure "vitamin-like" substances, which he thinks play the same rôle in plant nutrition that vitamins do in that of animals. Auximones are, therefore, to be included in the "nutrilites" of Williams.

Since they stimulate the nitrifying and nitrogen-fixing bacteria in the soil and at the same time depress the rate of denitrification, it has been suggested that the auximones are important in the synthesis of complex nitrogen compounds. At all events, bacterized peat seems to contain some growth-promoting substances, which are beneficial to various seed plants including the common farm crops; but to what extent it can be used under field conditions is one of the problems to be solved. How these auximones operate is also still an open question, but it seems certain that some plants do produce these auximones which are at least useful to other plants if not absolutely necessary.

It would not be surprising in the light of these investigations if future researches showed that the auximones are either provitamins or else bear the same relation to plant nutrition that the vitamins produced by plants bear to the nutrition of animals, as suggested by Breazeale; and the similarity between the relation of bios to yeast and that of auximones to *Lemna* is rather striking. Like bios (and unlike vitamins) the auximones are not made sufficiently rapidly by the higher plants to be of much use, but must be obtained from the culture solution.

These substances may be more necessary for green water plants than for land plants because the former live normally in an environment where there is never any question of their lack. The water plants have thus lost the power to manufacture their own vitamins, while land plants not so favorably situated have had to shift for themselves. But it should be added that Clark (1924–

1931) grew *Lemna* for over thirty generations or about five months on a purely mineral nutrient solution without the aid of any organic matter; and Wolfe (1926) and others have suggested that the favorable results of Bottomley were due to the fact that he started with improperly balanced solutions. The addition of the organic matter merely restored this lack of balance, and the plants grew better because of the improvement of the general nutrient situation rather than because of any specific effect of the organic matter added. Thus, even in the case of water plants, there is still doubt as to their needs for "auximones."

QUESTIONS

1. Give the derivation of the words, auximone, hormone, and vitamin.
2. Distinguish between hormones and vitamins; hormones and enzymes.
3. What are the endocrine gland secretions?
4. What structural advantages which favor hormone activity do animals have over plants?
5. Which is more nutritious, white bread or whole wheat bread? Why?
6. Compare animals and plants in their ability to manufacture auximones, hormones, and vitamins.
7. Why do children feel the effect of a deficient diet sooner than adults?
8. What is the difference between xenia and metaxenia?
9. What are vitamins B_1 and B_2?

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CHAPTER XVIII

DIFFUSION OF GASES

> They have their exits and their entrances.
> —As You Like It.

We have seen that, of the various materials which the plant needs to carry on efficiently its various activities, two—oxygen and carbon dioxide—come as gases from the air. The oxygen is necessary in the release of energy in the process of respiration, while the carbon dioxide, as has been emphasized already many times, is combined with water to form the carbohydrates, which, in turn, form the basis of the other products synthesized by the plant. During photosynthesis, the oxygen given off is so much greater than that required for respiration that probably no oxygen enters the cells containing chlorophyll at this time. The other cells must have oxygen continuously and, during periods of darkness, even the chlorophyll-bearing cells must, of course, take in oxygen from the outside.

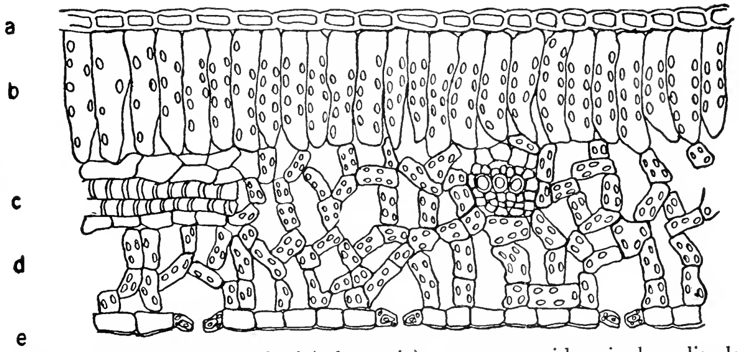

Fig. 9.—Cross section of a leaf (schematic). a, upper epidermis; b, palisade tissue; c, vein; d, spongy parenchyma; e, lower epidermis with stomata.

Structure of the Leaf.—Plants are built to permit an exchange of gases and to facilitate the free circulation of air with its contained oxygen and carbon dioxide. If we refer to the drawing of the cross section of a leaf (Fig. 9), it will be seen that the cells are in contact with air spaces, which connect with each other and with the stomatal openings. Even the cells buried most deeply in the leaf are thus seen to be in direct communication with the outside air, with the result that carbon dioxide can reach them freely.

Lenticels and Air Channels.—The cortex of the stem contains many living cells and is in similar contact with the outer air through the lenticels, which permit gases to diffuse in and out. Water plants, such as the water lily and lotus, have special air channels which connect the leaves floating on the surface with the stem lying at the bottom of the stream or lake. These air channels are so large that they can be easily seen with the naked eye. What child who knows them has not used them for soda straws or for blowing bubbles? In marshes are found plants like the cat-tail (*Typha*) and umbrella rush (*Cyperus*), which also have large air spaces connecting the parts above the soil with the roots growing in their muggy, airless environment. The underground portions of plants like the bald cypress (*Taxodium*) and the water willow (*Jussiæa*) which grow in swampy, poorly aërated soils have special outgrowths which come up into the air from the roots. These structures have spongy tips, which readily permit air to enter and thus act like ventilation pipes conducting air to the regions below. The pneumathodes of plants like the oil palm can be induced by excessive moisture and serve a similar purpose. The living cells of the roots are in this way kept in constant connection with abundant sources of oxygen.

Work of Molisch and Wiesner.—While the gases may diffuse freely into the intercellular spaces between the cells, that does not explain how they get into the protoplasm. Between the air chamber and the interior of the cell are two barriers—the cell wall and the plasma membrane—which must be passed. The most important work on the movement of gases through cell walls has been done by Wiesner and Molisch (1890) who experimented with various kinds of tissues fastened to the end of a glass tube. The tissue was sealed to the tube so that the margins were airtight and was supported in such a way that no cracks or breaks would occur. The tube was then partly or entirely filled with mercury, and the open end was closed with the finger while the tube was inverted and the lower end placed in a vessel of mercury. After the tube was thus arranged in an upright position, the height of the mercury column was measured and the time noted for the mercury to drop in the tube when the upper surface (with the membrane) was exposed to air containing the normal amount of gases. In this way, after many experiments carried on with various kinds of moist and dry membranes, Molisch and Wiesner came to the following conclusions:

1. The cell walls of both living and dead cells, whether wet or dry, do not permit gases to go through under ordinary pressures. A piece of birch bark 0.09 mm. thick thus supported a column of mercury 40 cm. high for two weeks without any change in the height of the mercury column.

2. Protoplasm and cell sap are also impermeable to the streaming of gases, which shows the necessity for the air passages previously mentioned.

After thus showing that *streaming movements* were impossible through cell walls and protoplasm, further experiments were continued to test the amount of *molecular diffusion* through similar tissues. In these experiments the tubes were partly filled with the gas to be tested and the rate of outward diffusion was measured by the rate of the rise of the mercury column in the tube (Fig. 10). From these experiments, they reached the following conclusions:

1. Gases diffuse through cell walls only in solution in the water which the wall has imbibed. If intercellular spaces are present, they facilitate the movement up to the cell wall, but the actual diffusion through the wall is in solution in the water of the wall.

2. The more water a wall contains, consequently, the more rapid is the passage of gases through it. In the potato periderm studied, the carbon dioxide diffused through the dry membrane only enough to cause a rise of 5 mm. of mercury, while in the wet periderm, the mercury rose 40 mm. during the same period of thirty days. This explains why water plants like *Elodea*, algæ, etc., are so readily permeable to gases and permit of such rapid diffusion.

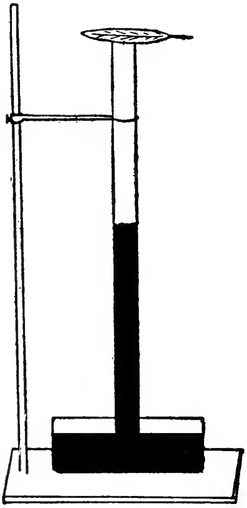

FIG. 10.—Illustrating the method of the Molisch and Wiesner experiments.

3. Even dry walls if they contain cutin or suberin, i. e., the fatty materials which characterize the outer layer of many plant

cell walls, will permit the passage of oxygen and carbon dioxide. This is extremely important, because it means that in dry weather and under conditions which tend to close the stomata, some oxygen can get through to the tissues beneath and the cells will not be suffocated. The plants will not dry out and yet at the same time will not die from lack of oxygen.

These investigators have thus shown that the carbon dioxide diffuses through the cell walls in solution in the water which impregnates them. Before it can get into the cell from the intercellular spaces it must first dissolve in the water of the cell wall and then diffuse through into the cell in solution. The water combines with the carbon dioxide forming a three-phase system of water, carbonic acid, and carbon dioxide. As the carbonic acid is used up inside the cell, more carbon dioxide diffuses in from the outside, and thus the supply is maintained.

Work of Blackman.—The third conclusion given above naturally leads to the question of the proportion of the carbon dioxide which finds its way into the intercellular spaces of the leaf through the stomata and the proportion which enters through the cuticle. To answer this question Blackman (1895) devised an apparatus by which gases could be led into a small chamber clamped to the side of a leaf. A gas of known composition was passed through the chamber and then analyzed again on leaving to see whether it had been modified by the gaseous exchange of the leaf. Experiments with leaves having stomata on both sides and those with stomata on only one, showed that the exchange which accompanies respiration occurred only through the surface where the stomata were. For example, the oleander (*Nerium*) gave out 0.065 g. of carbon dioxide from the lower surface and only 0.002 g., or less than one-thirtieth as much, from the upper side.

That the exchange during photosynthesis also takes place through the stomata and not through the cuticle, can be shown very easily by covering the lower surface with vaseline or wax, which thus plugs up the stomata. Under such conditions, as was shown by Mangin (1887), very little photosynthesis occurs, although it is not completely stopped. That the stomata determine largely the entrance of carbon dioxide is also shown in the case of the water plantain (*Alisma*). Here the lower surface has 74% as many stomata as the upper and absorbs 68% as much carbon dioxide.

Work of Brown and Escombe.—Brown and Escombe (1899–1900) then interested themselves in the question of the absorption of carbon dioxide through the stomata, and studied especially the question of rate. They worked on the *Catalpa* leaf, which has stomata only on the under side, and found that, under favorable conditions, one square meter of leaf surface absorbed 700 c. c. of carbon dioxide in an hour. They then computed the rate of absorption of each molecule of the gas and learned that the absorption in the leaf was only half that exhibited by a free surface of sodium hydroxide (NaOH) of the same area. But since the carbon dioxide goes in only through the stomata, one must compare not the entire leaf surface with the free surface but only the stomatal area. This is about one-hundredth the area of the leaf, so that the absorption through the stomatal areas is then not *half* that of a free surface but *fifty times* as great!

This led to the following experiment: Solutions of NaOH were placed in test tubes covered with thin perforated plates. Different plates had openings of different diameters, and the rate of diffusion was compared with the areas of the openings and their diameters. The following table gives some of the results:

Diameter of opening in mm.	*Ratio of opening areas*	*Ratio of diameters*	*Ratio of amounts of CO_2 entering*
22.70	1.000	1.000	1.00
6.03	0.070	0.260	0.26
3.23	0.023	0.140	0.16
2.12	0.008	0.093	0.10

This shows that the rate of diffusion through the small pores into the NaOH is a function not of the *area* of the openings but of their *diameter.* Thus the largest opening has an area more than a hundred times that of the smallest and a diameter only slightly more than ten times as great. The gas diffuses through the smaller opening, however, not one-hundredth as rapidly but one-tenth. It follows, therefore, that if a vessel of sodium hydroxide were covered with a perforated plate with very small openings properly spaced, the amount of carbon dioxide passing in would be as great as though the surface were entirely free and there were no plate present at all, even though the total area of the openings is considerably less than the total area of the surface.

Further experimentation showed that diffusion was most rapid when the distances between the openings were ten times their diameter, which is approximately the proportion existing between the area of the stomata and their distance apart. This means that the diffusion into the leaf is as great when the stomata are open as it would be if no epidermis were present and the carbon dioxide could diffuse in freely to the intercellular air spaces! Analyses of gases in the intercellular spaces have supported this conclusion since they show that the gases here have the same composition as the outside air.

Laws of Diffusion.—The rate of diffusion of various gases into the intercellular spaces follows the ordinary law of diffusion, i. e., the velocity varies inversely with the square root of the density. Thus oxygen, which is sixteen times as dense as hydrogen, diffuses only one-fourth as fast under the same temperature and pressure. The diffusion from the intercellular spaces into the cells does not depend upon the density, however, because one is here dealing with *dissolved* gases. In this case the rate of diffusion depends upon the solubility of the gas in the watery liquid through which it passes. If it were a question of the density, carbon dioxide would enter the cells more slowly than oxygen or any of the other common gases in the air, because it is the densest of them all; but, since it is a question of solubility, it enters more quickly than the others. Carbon dioxide is very soluble in water and thus, in spite of the small amount present in the air, it is absorbed by the plant in sufficient quantities to produce the luxuriant vegetation with which the land surface is in most places covered.

Carbon dioxide diffuses from the outside air into the stomata, then, according to the same laws which regulate the diffusion of gases everywhere; and the presence of the leaf cuticle, as shown by Brown and Escombe, is not a hindrance. Diffusion operates freely according to the gas laws, which may be illustrated by a very simple experiment.

Let us suppose a vessel made of palladium, holding one liter, is placed inside another vessel of glass which holds two liters (Fig. 11). Both vessels contain manometers for measuring the pressure of contained gases. The smaller vessel contains nitrogen and the outer vessel is filled with hydrogen. At the beginning of the experiment both gases are under one atmosphere pressure, so that the mercury in both manometers shows the pressure outside and inside to be the same.

Now palladium is permeable to hydrogen and impermeable to nitrogen; the former can pass through while the latter cannot. When this system is permitted to stand for some time, what changes will take place in the original equilibrium? Experiment shows that the hydrogen will diffuse through the palladium into the nitrogen until the hydrogen is evenly distributed throughout the entire system At the end there will be just as much hydrogen in the inner box as in the outer one. The nitrogen, unable to pass through the palladium, remains inside. Consequently after diffusion has been complete and equilibrium has been reached, the manometer on the outer box will register only a half atmosphere of pressure, while the one on the inside will register one and a half atmospheres. In other words, *each gas exerts its own partial pressure and each acts independently of the other.*

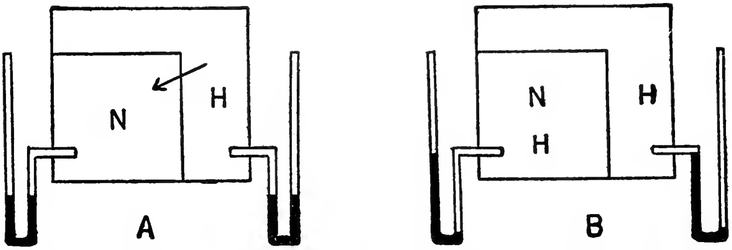

FIG. 11.—Illustrating diffusion. A, at the beginning of the experiment; B, at the end after equilibrium is reached. The hydrogen is evenly distributed throughout the system, having passed through the palladium wall.

A parchment membrane soaked with water, separating carbon dioxide and oxygen, with the oxygen on the inside, would show a similar result. The water membrane is much more permeable to carbon dioxide than to oxygen because of the greater solubility of the carbon dioxide in water. It would therefore pass in more freely than the oxygen would pass out.

These examples explain why there is a continuous stream of carbon dioxide passing into the leaf and into the cells. As the carbon dioxide is used up inside the cell, its partial pressure is less inside than outside and consequently more comes in to take its place. The oxygen which is present in greater quantity per unit of volume on the inside than on the outside will diffuse outward for the same reason. *Each gas moves independently of the others from the place where its partial pressure is the greatest to the place where it is the least.*

These laws, which the movement of gases obeys, should be kept well in mind, because we shall see in the next chapter that the movements of liquids and solids are subject to the same laws.

QUESTIONS

1. Why are leaves less variable in thickness than in area?
2. Why is the palisade tissue on the upper side of the leaf and the spongy tissue on the lower?
3. Why does washing the leaves of plants generally improve their condition?
4. Which suffer most from coal dust in the air,—evergreens or deciduous trees? Why?
5. Why does the addition of carbon dioxide to the *air* benefit the plant?
6. Is carbon dioxide more soluble in warm or cold water? What effect has this upon life in the arctic regions?
7. Would it be of any advantage if the stomata were more numerous?
8. If ordinary air were separated from a mixture containing half carbon dioxide and half oxygen by a water membrane, which gas would diffuse more rapidly into the air? Why?
9. Dangeard reported that he had kept leaves of *Aucuba* in a vacuum for 6 months. How is this explained? Were the leaves kept in the light or dark?

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CHAPTER XIX

OSMOSIS AND IMBIBITION

> They shall not pass.
> —GENERAL PÉTAIN.

Discovery of Osmosis.—As early as 1827 Dutrochet noticed the escape of zoöspores from the sporangia of some of the algæ and accounted for it upon a physical basis. He formulated the hypothesis that inside the sporangium were "water-attracting" substances which increased the entrance of water to such a point that the sporangium wall was stretched and then ruptured. He had seen that when an animal bladder is filled with a salt or sugar solution and then placed in pure water, the membrane is burst by the entrance of water, and he explained (correctly) the two phenomena in the same way.

Work of Pfeffer.—Because these observations of Dutrochet were the first in this subject, he is given credit as the discoverer of osmosis, but since his day an enormous number of workers have contributed to our knowledge of this important process. The first to carry on extensive investigations of prime importance was the German plant physiologist, Wm. Pfeffer (1877), who prepared special membranes for the study of the subject. It will be noted that one of the requirements for the successful observation of osmotic pressure is a membrane which is differentially permeable, i. e., which will let the solvent (water, alcohol, etc.) pass through but which will not let the solute (salt, sugar, etc.) penetrate. When the membrane is absolutely impermeable to the solute, it is said to be *semipermeable* but such membranes are rare, so that the term *differentially permeable* is preferable. This means that the membrane lets the solvent penetrate much more easily than the solute.

Membranes of parchment paper, bladder, and collodion are all good for the study of osmosis, but Pfeffer prepared precipitation membranes which are only slightly permeable to the solutes and, for this reason, are exceptionally advantageous. His method was to take a porous clay cylinder similar to those employed in making dry cells and to fill it with a solution of copper sulphate ($CuSO_4$). The whole is then placed in a solution of potassium ferrocyanide [$K_4(FeCN)_6$]. Since the cylinder is porous, both liquids penetrate the wall and meet in the middle of it where a membrane of copper ferrocyanide is precipitated:

$$K_4Fe(CN)_6 + 2CuSO_4 = Cu_2Fe(CN)_6 + 2K_2SO_4.$$

Copper ferrocyanide is a differentially permeable membrane. It will permit water to pass through, but not salts in solution. If such a cell is filled with a sugar solution, closed at the top, and supplied with a manometer, an increase in the height of the mercury column will be observed as soon as the cell is immersed in water, thus indicating an increase in the internal pressure. Such an instrument is called an *osmometer*, and with it Pfeffer and his students obtained the following results using cane sugar:

1. At 15° C.

Conc. of sugar	*Pressure (cm. of mercury)*
1%	53.2
2	101.6
4	208.2
6	287.7

That is, the osmotic pressure developed within a solution varies directly with the concentration.

2. A 1% solution.

Temperature	*Pressure*
6.8°	50.5
13.7	52.5
22.0	56.7

That is, the osmotic pressure within a solution increases with the temperature.

3. A 6% solution at 15° C.

Membrane	*Pressure*
Animal bladder	14.5
Parchment paper	29.0
Copper ferrocyanide	287.7

That is, the pressure derived varies with the membrane used because some membranes are more permeable than others.

Osmotic Pressure Similar to Gas Pressure.—Van't Hoff (1887) studied these results carefully and, after comparing them with the results previously obtained in studies made on the pressures in gases, he concluded that the dissolved substances were giving pressures in the same way that a gas exerts a pressure. In other words, he came to the conclusion that osmosis was a molecular phenomenon comparable to the pressure exerted by gases when confined. The results obtained by Pfeffer, as shown above, can be nicely interpreted in this manner. Doubling the concentration doubles the number of molecules and hence doubles the pressure. Increasing the temperature increases the pressure; and Van't Hoff showed that, as in gases, the osmotic pressure is proportional to the *absolute* temperature. He further showed that the osmotic pressure which the majority of substances give when dissolved in a liquid, is the same as that exerted by a gas at a given temperature with the same number of molecules in a given volume. To illustrate: At normal temperature and normal pressure (one atmosphere) a gram molecule (the molecular weight in grams) of a gas occupies a volume of 22.4 liters, so that, if the above law holds in osmosis, a gram molecule of sugar dissolved in 22.4 liters of water should give an osmotic pressure of one atmosphere. Sugar has a molecular weight of 342. Hence 342 grams of sucrose dissolved in *one* liter of water should give 22.4 atmospheres of pressure. A 1% solution would then give under these conditions a pressure of about 0.69 atmosphere. The results found in various ways by experiment show a 1% solution to give 0.62–0.71 atmosphere. That found by Pfeffer was 0.68 atmosphere, which is seen to be in very close agreement with the calculations made on the basis of the gas laws. This example is sufficient to show the justification of Van't Hoff's view.

Plasmolysis.—The effect of different substances on osmosis was tested by de Vries, the famous Dutch botanist (to whom we also owe the mutation theory of evolution), who used living plant cells instead of artificial membranes to answer this question. It will be remembered that the cell, on the outside nearest the cell wall and on the inside nearest the vacuole, possesses a plasma membrane which is differentially permeable, and it is these membranes which are of prime importance in the osmotic phenomena of the cell rather than the cell wall, which is only slightly differentially permeable. When a cell is placed in a solution of pure water, the water enters and causes the cell to distend. The protoplast is pushed firmly against the cell wall, and the cell in this water-gorged condition is said to be *turgid.*

If, on the other hand, the cell is placed in a strong salt or sugar solution, water leaves the cell. When the amount in the vacuole reaches a certain minimum, the protoplast withdraws from the cell wall and becomes balled up in the middle of the cell, leaving a space between it and the cell wall. These changes are especially easy to observe in cells with colored cell sap such as the epidermal cells of the primrose (*Primula*). In this condition the cell is *flaccid* or *plasmolyzed* and the state of the cell resulting from this excessive exosmosis is called *plasmolysis*. If the osmotic pressure inside the cell is greater than that outside, water will enter the cell; while if the greater osmotic pressure is in the liquid on the outside, water will leave the cell; and if the osmotic concentration is the same outside as inside, no change in the movement of the water will be observed.

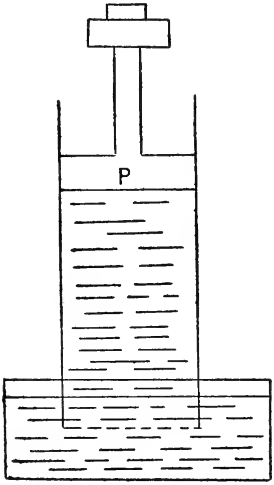

FIG. 12.—If the glass tube is closed by a semipermeable membrane with a 1% sugar solution on the inside and pure water on the outside, then a force of 10.35 lbs. is required to keep the piston, P, from rising, i. e., from being forced up, if it has an area of one square inch.

Solution Osmotic Pressure.—By solution osmotic pressure is then meant the pressure which would be exerted by a liquid if it were confined in a vessel and separated from pure water by a membrane which is absolutely semipermeable, i. e., which lets water enter freely but prohibits the solutes from passing out. Thus we say that a 1% sucrose solution has an osmotic pressure of 0.69 atmosphere, because it is capable of exerting that much pressure per square inch when placed in a container which is permeable to water and not to sugar. This can be illustrated further by supposing the sugar solution to be in a cylinder at the top of which is a piston. The bottom (Fig. 12) is closed by a semipermeable membrane. If the cross section of the chamber has an area of one square inch, it would take 0.69×15 lbs. or 10.35 lbs. to keep the piston from being forced upwards.

If two solutions have the same osmotic pressure they are said to be *isotonic*. If they are different, the lower one is said to be *hypotonic* in reference to the other and the higher one is *hypertonic* in reference to the lower.

Hydrostatic Pressure.—Instead of measuring the osmotic pressure directly, we measure in the case cited above the *hydrostatic* pressure which is developed by the osmotic pressure. A sugar solution in an open beaker possesses osmotic pressure, but this is not measured by the methods thus far described until this osmotic pressure is changed into hydrostatic pressure by separating it from pure water by a semipermeable membrane, when the osmotic pressure, now transformed into hydrostatic pressure, becomes noticeable. This distinction should be kept in mind.

Isosmotic Coefficients.—Let us return to the work of de Vries. By the plasmolytic method, he determined what solutions were isotonic with the cell by measuring the concentration just necessary to cause the first visible contraction of the protoplast from the cell wall; and with the use of 0.1 molar solutions of various sugars and salts (which therefore contained the same number of molecules) he found that the substances used fell into four groups according to the pressure developed, these pressures being 0.066, 0.100, 0.133, and 0.166 atmospheres, which are to each other as 2:3:4:5. In the first group, as shown in the following table, were sucrose and glucose; in the second, potassium nitrate and ammonium chloride, while in the last group was potassium citrate. These numbers (2, 3, 4, and 5) were termed the *isosmotic coefficients* and represent the relative osmotic pressures which are developed by solutions with the same number of molecules.

Substance	*Chemical formula*	*Isosmotic coefficient in round numbers*
Sucrose	$C_{12}H_{22}O_{11}$	2
Glucose	$C_6H_{12}O_6$	2
Glycerin	$C_3H_8O_3$	2
Potassium nitrate	KNO_3	3
Ammonium chloride	NH_4Cl	3
Potassium sulphate	K_2SO_4	4
Magnesium chloride	$MgCl_2$	4
Potassium citrate	$K_3C_6H_5O_7$	5

It will be observed from this table that substances like glucose and sucrose which have very different molecular weights exert the same osmotic pressure when the same number of molecules are in the solution. They thus follow the gas law and agree with the explanation of osmosis as given by Van't Hoff. But the other substances,—the salts,—seem to be exceptions. If they followed the Van't Hoff rule, they also should give the same osmotic pressure.

Ions Act Like Molecules.—This difficulty was explained by Arrhenius, and, in fact, the osmotic pressures developed by salts are considered among the strong supports of the ionic theory of solution connected with the name of this great Swedish chemist. According to Arrhenius, salts (which are composed of a base united with an acid) partly dissociate in solution to form the basic and acid *ions* (Chap. XI). NaCl, e. g., partly dissociates to form sodium and chlorine ions. In a solution there are present not only molecules of sodium chloride but also ions of sodium and chlorine. In the solutions used by de Vries about one-half of the salt thus dissociates, so that the number of osmotically active units would be increased 50% over what it would be if no ions were present but only molecules. This then explains very beautifully why the isosmotic coefficient for KNO_3 and NaCl is 3 instead of 2. Likewise in the case of potassium sulphate if half the molecules are dissociated, instead of two molecules of potassium sulphate there are one of the salt, two ions of potassium (since the salt is divalent), and one ion of the sulphate. The isosmotic coefficient is thus twice that of materials like sugar which do not dissociate (nonelectrolytes) or 4. The degree of dissociation varies with the concentration, and consequently the figures employed here cannot be used for higher concentrations. In general, the more dilute the solution the higher the percentage of dissociation.

Osmosis and Diffusion.—This explains in part some of the results obtained in the experiments with osmometers, but there are still many questions which here can be touched upon only briefly. First, why does the water pass into the solution? One answer has been that the surface tension of the solution is different from that of the water outside and, in order to equilibrate the two surface tensions, water must pass in according to the laws of thermodynamics. Others have suggested that the hydrostatic pressure which develops when the solution is separated from water by a semipermeable membrane, is the difference between the normal osmotic pressure inside the osmometer and the atmospheric pressure on the surface of the liquid outside. The water goes in until this difference is turned into hydrostatic pressure and the two forces are balanced. However, the most popular explanation of the phenomenon and the one easiest to comprehend, after what has been said in the previous chapter, is that based upon the laws of diffusion. There we saw that substances diffused independently of each other and according to their own partial pressures. We can view an osmometer in the same way. On the inside we have, e. g., a 40% solution of sugar. That means there is only a 60% solution of water on the inside. On the outside, however, there is a 100% solution of water. In other words, the water is more "concentrated" (to use a term which is generally used for the solute only and not for the solvent) on the outside than on the inside, and so it moves from the place where it is the more concentrated (has a higher partial pressure) to the place where it is the least. This movement continues until the difference between the two solution pressures is balanced by the hydrostatic pressure exerted by the column of liquid. If the liquid inside the osmometer is rich in solutes, i. e., poor in water, the column will hence be higher than if the solution inside is relatively poor in solute and rich in water. The salts or other solutes also tend to diffuse out, and do so as much as the membrane will permit.

The Membrane.—The second question to consider is how the materials pass through the membrane. Some think that the solvent dissolves in the membrane (or *vice versa*) and passes through in this fashion. Others have suggested that reversible compounds are formed which tie up the solvent with the membrane on one side (the outside) and release it on the other. In certain cases such as the passage of alcohol, pyridine, etc., through rubber membranes there may be some truth in the solution and reversible compound theories, but most of the work can be explained more easily on the assumption that the membranes are porous. These pores of a certain size let water pass through but not the larger molecules of sugar, salt, etc. In very dilute solutions electrical phenomena play a part, but in the study of elementary plant physiology this type of osmosis can be neglected.

A third question is why the water stays in after it gets in. Since it is free to pass out, why doesn't it do so? One answer is that the particles inside attract the water and hold it so that once inside it is not free but is bound up with the particles present. Since they cannot pass out, neither can the water which is bound to them. A second explanation is that the osmotically active substances inside act as a trap and block the way. Molecules of water can enter freely but once inside they are hindered from passing out by the sugars, salts, etc., in the path. Any theory which is acceptable must explain why the hydrostatic pressure developed varies with the number of molecules or particles of solute present. Each of the above theories does this. If more water-attracting particles are present, more water will be held and the pressure will vary accordingly. If, on the other hand, the trap theory is accepted, here also the more particles present, the more would be in the way to hinder the outward passage of the water and the greater would be the hydrostatic pressure developed.

However, in the light of the diffusion hypothesis favored above, it may not be necessary to assume either of these two hypotheses. If diffusion can explain the entrance of the solvent, and if the water diffuses in because there are more molecules outside than inside, then no further hypotheses may be needed. The more sugar inside, the less water, and the more must flow in before an equilibrium results. This will also explain why the pressure varies with the number of molecules of solute present.

Osmosis and Plants.—To return to the plant, we need now only to apply these principles to the conditions found there. Every cell is bounded by a differentially permeable membrane which is generally more permeable to water than to solutes. This membrane is not the cell wall, which plays only a minor rôle in these phenomena, but the plasma membrane. Other membranes are present in the cell, such as the tonoplast, the nuclear membrane, the membranes bounding the plastids, etc., all of which exhibit these same properties, which are summed up in the term *differentially permeable.* These membranes, however, all differ in one important essential from the parchment, bladder, etc., already described, viz., in the fact that they *are alive* and that *their permeability changes.* Some of the factors which determine these changes in permeability have already been mentioned (Chap. XI).

Every substance which enters the plant must enter in solution and obey the laws of diffusion. If the plasma membrane is permeable to KCl and, if there is more KCl in the soil water than inside the root hairs, this salt will diffuse in. Because of the formation inside the plant of osmotic substances such as sugars, etc., there is generally a higher osmotic solution pressure inside the cell than outside, with the result that water enters. There is thus maintained a condition of hydrostatic pressure inside the cell known as *turgor.* For this to be manifested there must be (1) a difference in the osmotic pressure within and without the cells and

(2) a differentially permeable membrane. As we have seen, these conditions are both met, the first by the more concentrated cell sap as compared with the watery solution in the soil and the second by the plasma membrane. As Thoday (1918) has pointed out and earlier Ursprung (1916), if P is the osmotic pressure within the cells and T the turgor pressure, then the amount of water entering the cell at any given moment depends upon P–T. While the turgor pressure increases owing to the intake of water, the osmotic pressure decreases as the solution becomes diluted. This value of P–T is known as the *suction force.* Consequently, in considering the possible exchange of water between two adjacent cells, one must consider both the osmotic and the turgor pressure.

The osmotic pressures found in plant cells are sometimes very high. De Vries found the following values for the expressed juice:

Potato leaves (*Solanum*)	5.5 atmospheres
Mountain ash berries (*Sorbus*)	9.0 "
Beetroots (*Beta*)	21.0 "

While plant cells in general range from 5–10 atmospheres, desert plants have been found to possess pressures exceeding 100 atmospheres. Some of the molds when grown in concentrated solutions develop pressures as high as 150 atmospheres, and *Atriplex confertifolia* growing near Great Salt Lake has an osmotic pressure of 153 atmospheres! This is the world record for Angiosperms. Even 10 atmospheres means a pressure of 150 lbs. per square inch, and it is not to be wondered at that plants can split rocks, lift sidewalks, and perform similar feats of strength.

Absorption by Plants.—Most of the water and salts absorbed by plants are taken in by the roots, but some water is absorbed also by aërial parts, especially leaf hairs and other uncutinized portions of the aërial organs. Such absorption takes place in *Eriospermum*, *Massonia*, and *Gethyllis* by the leaf hairs (Marloth, 1926), and in the Australian salt bush (*Atriplex*) by the uncutinized leaves, which are especially rich in accumulations of sodium chloride.

The chief absorbing region of the root lies directly back of the root cap and is enlarged many fold by the formation of the root hairs, the suction force of which plays the chief rôle in determining whether water will be absorbed under a given set of osmotic conditions. The intake of salts is *largely* determined by the laws of diffusion. For this reason a plant often takes up salts which have no special value to it if they (or their ions) are in higher concentration in the surrounding soil solution and if the cell membranes are permeable to them. Thus peas, oats, radishes, and buckwheat all increase in iodine content when potassium iodide is added to the culture solution even though these ions may be of no value to the plant (Kelly and Stuart, 1928). We have also seen (Chap. X) that plants contain many elements that are of no known value and which they can get along perfectly well without.

But the entrance of salts and ions is not a question of physical diffusion only. In some plants materials seem to be taken in when there is already a larger concentration inside the cell than in the liquid outside. Hoagland and others found in the alga, *Nitella,* that the concentrations of Na, Ca, Mg, Cl, SO_4, and PO_4 were all higher inside the cell sap than in the surrounding water. A possible explanation for this is that the ions (or molecules?) are absorbed and then combined in an "inactive" state so that more can then enter. On the other hand, it is possible that under certain conditions (very imperfectly understood) the protoplast may actively absorb certain ions against a concentration gradient. This means that the laws of diffusion do not operate as with a dead substance but that the living protoplasm plays an active rôle in determining what materials shall or shall not enter.

These conclusions agree with the known facts that two plant species growing in the same soil will absorb different elements in different amounts according to the "natural needs" of the particular species. This specific character of the protoplasm may be inherited the same as any other character as in the case of Egyptian cotton, which absorbs chlorides more readily than Upland cotton; while the latter absorbs sulphates more easily than does the Egyptian. When hybridized, this character blends in the first generation, but in the second generation shows a Mendelian segregation (Harris, 1924–1925).

Measurement of Osmotic Pressure.—The osmotic pressure is commonly measured in one of three ways. First, one can measure the hydrostatic pressure developed. This, including the plasmolytic method, is an indirect one and has already been described. Simpler methods which depend upon physical changes connected with the number of osmotically active particles in solution have been developed. One of these is the determination of the boiling point. The higher the osmotic pressure, i. e., the greater the number of particles in solution, the harder it is to drive off the water by heating. Consequently the vapor pressure will be less and the boiling point will be higher. The osmotic pressure can then be told by the elevation of the boiling point.

Similarly in freezing it is necessary to separate the water from the solute, and the more solute present the harder it is to freeze the solution, i. e., the lower must be the temperature. Consequently the depression of the freezing point is an index to the osmotic pressure, and this method is the one commonly employed with plant and animal liquids.

In conclusion, it may be briefly added that the osmotic properties of cells are among the most important since, along with the condition of the membranes through which the materials pass, they largely determine the movements of all substances from cell to cell within the plant.

Imbibition.—Osmosis has explained only how the water and solutes enter the living protoplasm. While this is of the highest importance, there yet remains something to be said of the method by which water passes through the dead parts of the cell such as the cell wall, through seed coats, etc. This is by *imbibition.* If we place water in a glass tube, the water which wets the glass will rise slightly in the tube to a height which varies with the diameter of the tube. The elements or molecules of the water *cohere* to each other and *adhere* to the glass. While the forces of adhesion are stronger than gravity, they are not strong enough to overcome the cohesive forces within the water itself, with the result that a small column of water is pulled up the glass. If, on the other hand, water and sugar are put together instead of water and glass, we find that the adhesive forces between the molecules of water and sugar are stronger than the cohesive forces of the sugar, with the result that the sugar is pulled apart and broken down into its constituent molecules. In other words it *goes into solution.* Here we have the two extremes. In capillarity, cohesive forces are much the stronger of the two; in solution, the adhesive forces get the upper hand. In imbibition, the forces are much more evenly balanced. The water is attracted very strongly by the particles which make up the cellulose cell wall, and the particles of the cellulose are also attracted by each other; but they are nevertheless forced apart by the water which is able to come in between them. When the water is imbibed, the walls are stretched and swell, but when dried out they go back to their original shape.

In imbibition we have to deal with the same forces that are present in the phenomena of solutions on one hand and capillarity on the other. Only here the two forces are much more nearly the same. The water is attracted with an enormous force resulting in very large pressures. It has been estimated that two cover glasses an inch square require several hundred atmospheres (2,000) to hold them together so firmly that no water can enter between them when placed along the edge. The forcing apart of the bones of the skull by inserting swelling peas and then adding water is a common practice in anatomical work. Boats have been known to split apart when leaks permitted the entrance of water to a cargo of rice or beans, as in the case of the Hapag motorship, Rhineland, in 1926.

Although, when water is imbibed, there is a change of volume owing to the swelling, the resultant volume is less than the sum of the water and the imbibing tissues. This means that there has been a compression along with the swelling. The result is the emission of heat, which can be easily measured.

What are the particles of the cell wall which are forced apart by the entering water? Some have thought them to be the molecules of cellulose, but others, led by Nägeli, have considered the particles to be *groups* of molecules which he called *micellæ*. These he thought were the units which played the chief structural and physiological rôles in the cell wall.

In conclusion, then, it must be borne in mind that imbibition and osmosis are the important processes which determine the entrance of water into the cell. The former is a phenomenon allied to capillarity and solution, and in plants is chiefly concerned with the dead tissues like the cell walls. Osmosis, on the other hand, is a diffusion phenomenon; it plays the more important part, and is concerned with the transfer of substances into and out of the living cells.

QUESTIONS

1. Apply the principles of osmosis to (a) the opening and closing of stomata and (b) the wilting and recovery of plant tissues.
2. Why do red beets which are boiled lose more of their coloring matter than unboiled ones?

3. Under what conditions will a dissolved substance pass through a membrane without any movement of water taking place?
4. Under what conditions will a dissolved substance pass through a membrane in one direction and water in another?
5. Which has the higher osmotic pressure,—a parasite or its host? Why?
6. Why do fruits sometimes split open in very wet seasons?
7. In plasmolysis, is the cytoplasm pushed or pulled from the cell wall?
8. A plant grown in water-culture solutions will remove most of the salts even when its roots fill only a small part of the bottle. Why?
9. Why does a concentrated spraying mixture sometimes kill the leaves of a plant?
10. Why are salt solutions commonly used on gravel walks to kill weeds?
11. Explain the action of salt and sugar as a preservative of meats and vegetables.
12. What are zoöspores and how do they escape from the zoösporangium?
13. What would you expect the isosmotic coefficient of magnesium sulphate to be?
14. Why does rain at pollination time commonly result in a small set of fruit?
15. Seeds generally have an osmotic pressure of 20–45 atmospheres. What "survival value" have these high pressures?
16. Would seed germinate more or less readily in a salt solution?
17. Epiphytes of the tropical rain forests often have a very low osmotic pressure (3–6 atmospheres) in their cells. Explain.
18. Harris and Lee (1930) found that the drouth resistance of sugar cane varieties in Hawaii was directly proportional to their osmotic pressure. Explain.

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CHAPTER XX

LOSS OF WATER

> You scarce could think so small a thing
> Could leave a loss so large.
> —G. MASSEY.

The plant requires, as has been already pointed out (Chap. IV), large quantities of water which are used in the manufacture of food. This water remains in the plant until the food is oxidized or broken down when it may be released; but, in addition, the plant needs water for other purposes. Water (1) is an essential component of protoplasm, (2) serves as a solvent for oxygen and carbon dioxide, (3) aids in the transportation of raw materials and foods, (4) has a high specific heat and absorbs much excess heat energy, and (5) maintains turgor in the living cells.

The average water content of some common plants is indicated in the following table:

Plant	*Per cent wt. of water*
Apples (fruit)	83
Beets	87
Cabbage	90
Carrots	85
Red clover (green hay)	71
White clover (green hay)	78
Corn (dry seed)	11
Corn (green fodder)	80
Cowpeas (green hay)	84
Cucumbers (fruit)	96
Grape (leaves)	69
Oats (dry grain)	11
Onions	88
Irish potatoes	79
Sweet potatoes	71
Pumpkins	93
Rice (grain)	13
Spinach	90
Timothy (cured hay)	42
Wheat (grain)	14

In addition to satisfying all these needs, the plant also requires large amounts of water to replace that lost by bleeding, through the action of glands, by guttation, and by transpiration, each of which causes of water loss will be discussed in turn.

Bleeding.—Bleeding, which was first investigated by Hales (1735), is the flow of water and cell sap with the included materials from cut and wounded plants. It is thus not a process which takes place under normal and healthy conditions. The amount of material which exudes differs with the plant, with the time of year, etc. The following table, taken from the work of Eckerson (1908), shows some of these variations:

Plant	*Mean quantity in c. c.*	*Days of flow*	*Mean pressure in atmospheres*
Begonia coccinea	168	29	0.858
Chrysanthemum frutescens	40	15	1.014
Fuchsia speciosa	99	23	1.246
Helianthus annuus	30	16	1.276
Lycopersicum esculentum	13	5	1.164
Pelargonium zonale	15.5	10	0.881

In some plants, such as the sugar maple, the southern pine, the rubber tree, and the Mexican agave, the bleeding is of great commercial importance. In these cases the exudate is collected and from it are obtained maple sugar, turpentine, rubber, and pulque. In the case of the maple, 3–7 liters may be derived from a tree each day during the sugar season; while the agave of Mexico and Central America may furnish 500 liters of sap during the season! After the plant has grown for ten to twenty years it sends up a huge flower stalk bearing many flowers and fruits, but just as this stalk is about to develop the bud is removed. Into the hollow thus formed the plant exudes 7–8 liters of sap a day for about two months. The record daily amount thus far reported is from the wine palm of Malasia (*Caryota*) which is reported to exude as much as 50 liters a day! Although this individual flow is exceptional, it has been calculated that from an acre of these trees about 14,000 gallons of exudate may be collected annually.

The conditions under which bleeding occurs are an abundant water supply and a limited normal loss by transpiration. The spring, when there is abundant water in the soil and the leaves are not yet developed, is hence the ideal season for such bleeding. This explains also why the removal of the top of the plant results in bleeding; the water supply is maintained below and the normal outlet above is removed. The amount of sap thus exuded may be far greater than the total volume of the roots, showing that water continues to enter the plant after the wound or cut has been made. A sunflower (*Helianthus*) bled 5,830 c. c. of liquid, while the total volume of the roots was only 3,370 c. c.

The pressure from below which results in bleeding is called *exudation* or *root pressure.* That such pressure exists can be shown by attaching a manometer to the cut stem as shown in Fig. 13, although it should be added that the pressure is not an index to the amount of sap which exudes. The pressure may be high and yet there may be only a small amount of exudate, as the previous table shows. This pressure is a manifestation of the turgor pressure in the cells below the cut, which is apparently connected with permeability changes in the cells, with the result that water is forced out of them into the vessels and up the stem; but just how this turgor pressure is connected with bleeding is still an unsettled question.

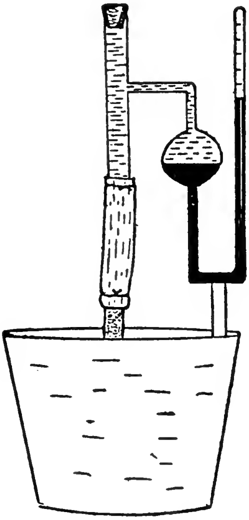

FIG. 13.—Arrangement illustrating one type of manometer for measuring root pressure.

Glands.—Secretion is the exudation from specialized cells called glands, and is a common occurrence in plants. In addition to water, it is possible to find in the secretions of various glands sugars, oil, digestive enzymes, etc., some of which, e. g., the enzymes, have obvious functions. This is a good nutrient medium for many fungi, and the organism which causes pear blight frequently infects the plant through this channel. The secretions from floral nectaries attract insects which bring about cross-pollination. Oily and resinous secretions may protect the plant from predatory animals. Some of these secretions may be only waste products. All, however, present the problem of how the cell, which is normally impermeable to the secretions, permits their escape. Does the turgor of the gland cell increase to such a point that the materials inside are forced out? Or are there intermittent changes in the permeability of the cell which permit the contents to pass outside? In the light of recent investigations the latter would seem the more probable solution.

Guttation.—Guttation is the escape of water in drops from *uninjured* plants. It generally occurs in leaves at the tips of the veins, where there are especial outlets called *water stomata* or *hydathodes.* These water losses occur under the same external conditions as bleeding, i. e., when there is an abundant water supply and when normal transpiration is checked by a humid atmosphere. These hydathodes thus seem to be safety valves which permit the excess turgor pressure to be reduced when it gets too high. Guttation may be easily observed by covering a young nasturtium (*Tropæolum*) or wheat plant with a bell jar and watering abundantly. In a short time, drops of water will be seen at the ends of the veins. These drops get larger and larger when they finally run down the leaf or drop off. This is often erroneously taken for dew, but may be distinguished from it by its special location. In these drops, plant disease bacteria often live and from there infect the plant. In some cases, e. g., the black rot of cabbage and the angular leaf spot of cotton, this is the chief method of infection. Artificial guttation can be produced by injecting the plant with water under pressure from the city water tap. When so attached to a supply of water under pressure, the guttation drops are soon observed at the usual places.

Normal guttation is best observed in the tropics where there is an abundant supply of water in the soil and the air is nearly always saturated with water vapor. In some plants, notably aroids, such guttation is extremely large. A good healthy leaf of *Colocasia antiquorum* has been known to yield over 110 c. c. of water a day, the water dropping at times at the rate of over 100 drops a minute (Duchartre).

The water pore or hydathode through which this water passes is an aborted stoma whose guard cells no longer function, with the result that it always remains open. Between this opening and the tracheids of the leaf is a layer of parenchyma cells called the *epithem*, which resembles the transfusion tissue normally surrounding the tracheids. In some cases the epithem seems to behave like a gland, actively secreting water out through the opening. In other cases it is merely passive, with the result that the composition of the water exuded is approximately that of the soil or culture solution. In the *Colocasia* mentioned, Flood (1919) found no epithem in the hydathodes, which may explain in part the high guttation rate.

Transpiration; Demonstration.—The losses thus far discussed are minor and unimportant compared with the loss through transpiration, by which is meant the loss through evaporation from the plant surfaces. That such losses do take place can be easily demonstrated:

1. If a plant is well watered and placed under a bell jar, care being taken to cover the pot and soil with oiled paper so that no water loss can take place from these sources, in a short time drops of water will be seen to collect on the sides of the jar, enlarge, and run down. These drops of water have passed off from the plant in the form of water vapor and condensed on the glass surface of the jar.

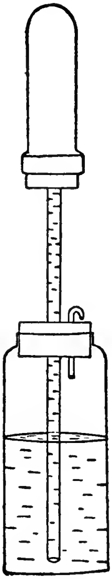

FIG. 14.—An atmometer. The unglazed shell and tube are filled with water and inverted in the glass jar which is also filled with water. What is the purpose of the small glass tube, and why is the upper end bent down?

2. Another easy and convenient way to demonstrate this water loss is to place a well-watered plant on the pan of a balance, taking the same precautions as before to check all evaporation except from the plant itself. After the pans have been balanced, it will be noticed that in a very short time the one holding the plant begins to rise as water is lost.

3. One may also note the rate of transpiration indirectly by means of the *potometer*. A branch is fastened in a vessel filled with water to which a horizontal glass tube is attached. As water evaporates more is drawn in from the vessel and consequently the meniscus at the end of the column of water in the tube shifts its position nearer the bottle. This is not an accurate method of measuring transpiration because it measures water *used* by the plant rather than water *lost*.

4. Another method much in use is the cobalt chloride one. In this case paper impregnated with cobalt chloride is placed in close contact with the leaf surface and the time noted for the blue paper to change to pink as water vapor from the leaf is absorbed.

Atmometers.—In transpiration studies it is often convenient to compare the plant with a dead evaporating surface, and, for this purpose, porous clay cylinders called atmometers (Fig. 14) are filled with water and connected with a water reservoir. These instruments have been much employed by Livingston and others who are interested in the evaporating power of the air, and who wish to compare the evaporation from purely physical evaporating surfaces with that from living membranes. Atmometers do not respond to changes in the environment in the same manner as plants, however, since the latter are alive and do not lose water in the same manner as dead membranes. The atmometers are, therefore, not *exact* indices to the behavior of plants under similar conditions.

Amount of Water Loss.—The amount of transpiration is enormous. Haberlandt has estimated that a single corn plant will transpire 30 pounds of water during the growing season. A single sunflower plant during its growing season may transpire 150 pounds, and for this reason sunflowers have been grown in swampy soils for reclamation purposes. It has been similarly estimated that land covered by grass or clover might evaporate 500–750 tons of water from each acre, that an acre of 40 apple trees 30 years old might lose during the same growing season 600 tons of water, and that an acre of corn requires four and a half tons of water for photosynthesis in addition to the 1,700 tons lost by transpiration. The average leaf under mesophytic conditions transpires in 3 months an amount equal to its area and a centimeter deep. The operators of greenhouses figure on 50 grams per square meter per hour by day and 10 by night, or 720 grams for each 24 hours.

The water needs of plants are thus seen to be extremely high. Various plants require different amounts of water because of their differences in size, environmental conditions, etc., but the actual amount of water transpired is not so important as the efficiency with which the water is used. This efficiency is determined by comparing the amount of total water transpired with the weight of the dry plant material which the plant produces. The quotient of these two is called the *water requirement* of the plant:

$$\frac{\text{weight of total water transpired}}{\text{total dry weight}} = \text{water requirement.}$$

Using this formula, durum wheats (grown in the dry-farming sections of northwestern U. S. A.) have a water requirement of about 400, while for common wheats the figure is about 600. This means that the former can make a gram of dry matter (or a pound) when only 400 c. c. (or 400 pounds, i. e., about 50 gallons) are supplied it, while the common wheats require about 50% more water to form the same amount of dry matter. Some common plants may be arranged in ascending order of their water requirement in northern Ohio as follows (Shantz): millet, sorghum, corn, sugar beet, barley, buckwheat, wheat, cabbage, cotton, potato, oats, rye, legumes, and flax. Wheat and oats have a water requirement about double that of millet, and legumes about three times as much. Such figures, however, are somewhat relative, since plants are generally somewhat less economical in their use of water when they have plenty of it, i. e., in a moist soil, the water requirement tends to be somewhat higher than in a dry soil (Yuncker, 1916).

Causes of Transpiration.—Why does this enormous water loss occur? The simplest answer is that the plant cannot hinder it. If one recalls the internal structure of the leaf (Fig. 9), it will be remembered that the cells of the mesophyll are thin walled, that they are soaked with water so as to permit the exchange of gases, and that they border on air spaces from which diffusion into the outer air is extremely easy. Under such circumstances, there is a high gradient between the amount of water in the cells and that in the air next to them. Consequently the cells *must* give off water. They are built for gaseous exchange, but such a mechanism which lets gases in and out must also permit the escape of water vapor. All structures must be a compromise, bringing both advantages and disadvantages, and nowhere is this better shown than in the leaf. A plant which is built for manufacturing food easily, is built, unfortunately, so that water can pass off easily. If plenty of water is to be had, this is not a disadvantage since there is no object in conserving it; but if water is scarce the plant is at a decided disadvantage, and, if it is to survive at all, it must grow slowly and conserve the water supply as carefully as possible.

Transpiration as a Function.—Transpiration used to be considered a "function," but it is now seen to be an evil and a hindrance to the development of the plant even though it may entail some minor advantages, among which have been proposed (1) the aid to the "circulation" of water in the plant, (2) the distribution of salts, and (3) the lowering of the temperature.

The transpiration water stream which is drawn through the plant as the result of the evaporation keeps the water coming in as fast as it is lost if there is an abundant supply, but since there would be no need for this replacement if it were not lost by transpiration, it is hard to see how this can in any sense be called a "function."

The effect upon salts is a more disputable one. It was once held that the transpiration stream swept along with it the salts in solution in the soil water and thus aided in their circulation through the plant. Although it would take many years for a molecule of salt to reach the top of a giant sequoia by simple diffusion, after the water gets into the vessels of the wood, there are comparatively few cell walls to pass through before it gets to the living cells of the leaf, and the transpiration stream certainly helps to further the rapid circulation in this open stream; but since the passage from the soil into the vessels depends upon osmosis and the general laws of diffusion, it is difficult to see how transpiration could increase directly the rate of entrance from the soil, owing to the fact that salts and water move independently according to their own partial pressures.

Schlösing (1869) grew tobacco under a bell jar and also free in the air. In the moist atmosphere under the bell jar, the ash was 13% of the dry weight, while out in the dry air, where there was plenty of evaporation, the ash was 22% of the dry weight. This would indicate that transpiration *does* increase the amount of salt taken in by the plant. Mendiola (1922) failed to confirm the results of Schlösing, and McLean found that in tropical rain forests the shade leaves, with a lower transpiration rate, had a higher mineral content than the sun leaves; but Muenscher (1922) found that when transpiration of barley was checked by lowering the illumination, the ash content was decreased. Likewise Haas found that in windy weather, when the transpiration of *Citrus* was increased, there was an increase of calcium salts in the leaves. If the transpiration stream hastens the movement of salts within the vessels it would seem only to be expected that this would remove the salt from the base of the plant near the roots, and according to the laws of diffusion more would then diffuse in. The accumulations of salt are known to be higher in the leaves than in any other plant organs (Chap. X), and this accumulation and storage in the leaves would then entail an entrance of more salts below. It is hence the conclusion of the writer that while it is not the *function* of transpiration to bring in more salts from the soil, as the plant would probably get enough salts without this, it is reasonable to expect that the entrance of more salts will be a *result* of transpiration.

As has been shown (Chap. IV), only a small amount of the energy which is absorbed from the sunlight is used to manufacture food. The rest, which is absorbed in the form of heat, would soon raise the leaf to so high a temperature that the protoplasm would be coagulated and killed if this surplus heat were not absorbed by the water present and evaporated. This results in a lowering of the temperature and, instead of being much warmer than the surrounding air, the leaf has about the same temperature or even a lower one. Although Clum (1926) reports the temperature always higher than the surrounding air, this may have been partly due to the use of the thermocouple which slightly wounds the tissue. Miller and Saunders (1923) found that in bright sunlight most leaves kept about the same temperature as the surrounding air, but alfalfa leaves even here were a trifle cooler. In the shade, however, the leaves were as much as 3° C. cooler than the surrounding air; and it has been noted also that leaves which are beginning to wilt may be as much as 6° C. warmer than normally turgid ones. When the sun shines brightly after a shower, leaves are commonly scalded for two reasons: first, the transpiration is checked by the high humidity of the air after the rain and, secondly, the little drops of water remaining on the leaves act like convex lenses of a burning glass and focus the light even more strongly than ever upon the leaf. The lowering of the temperature by transpiration, if such a lowering actually occurs, is of genuine benefit and, from the *functional* point of view, in this respect only can transpiration be called a function. Even here, as pointed out by Curtis and Clum (1926), the benefit is very doubtful.

Evaporation from the plant takes place mostly through the stomata of the leaves and the lenticels of the young bark. Wherever there are openings which permit the exchange of gases, through these water vapor is destined to pass out. A small percentage of water (5–10%) also evaporates from the cuticle, but this cuticular transpiration is nearly negligible in comparison with the amounts which pass off through the stomata.

Determining Factors.—The factors which determine transpiration are both external (outside the plant) and internal. The external conditions which affect transpiration are those which affect the evaporation from any moist surface. First of all is the *relative humidity* of the air. If the atmosphere is relatively full of water vapor, diffusion from the plant is retarded. In a saturated atmosphere as during rain, mist, or fog, there is practically no transpiration. As the humidity decreases from 100% to the 70% of a moderate day in mesophytic climates or to the 50% and less of desert areas, evaporation increases. As the humidity varies from day to day and hour to hour, the changes in transpiration run parallel.

Temperature likewise affects transpiration largely through its influence upon humidity. As the temperature increases, the relative humidity becomes less and transpiration increases. Increase in soil temperature also increases transpiration.

Barometric pressure is important only because of its relation to the vapor pressure and the ease with which water vapor can pass off into the air. The lower the pressure the more will be the transpiration. Although this is of importance only when comparing plants at low and high altitudes, on mountain tops where the low pressure is associated with low humidity and intense light, this may be a powerful factor in the distribution of plants. Whitfield (1932) found that transpiration decreased with altitude, but his data seem to show merely that the difference in barometric pressure was more than offset by the lower temperature and greater humidity, so that the net result was a decrease in transpiration.

Air movements affect transpiration through their influence upon the humidity. Clothes hung upon the line dry more quickly on a windy day since the layer of saturated air becomes removed by the wind as fast as formed. In a similar manner, transpiration varies with the amount of motion of the surrounding air.

The *chemical properties of the soil* affect transpiration. This effect may be due to quantity (concentration) of materials or to the quality (nature) of the materials present in the soil. A small amount of salts in the water has little effect upon transpiration, but, as the amount increases, there is a decrease which is probably due to the osmotic effect produced by the higher concentration of salts. Acids may accelerate while alkalies may retard transpiration. Specific salts may also exercise such inhibitory and accelerating effects.

Of the various external factors, *light* is one of the most important, partly through its action upon the stomata in connection with photosynthesis. Light tends to cause the stomata to open so that food may be manufactured (in a fashion described below), and when the stomata are in this open position more water escapes from the leaf. In addition to this direct light effect, there is an equally important *heat* effect, because the heat energy required to evaporate the water comes very largely from the light. Brown and Escombe estimated (1900) that a sunflower leaf on a bright day transpired 275 c. c. per square meter of leaf surface per hour, which requires 166,800 g. cal. of heat. Since the leaf area receives during this time a total of about 600,000 cal., it follows that nearly 30% of the total amount of energy received by the leaf is used to evaporate the water from it. Transeau made some calculations on corn and concluded that here about 45% of the total light energy was used up in transpiration.

The Wilting Coefficient.—The amount of water in the soil is, of course, an external factor of prime importance in determining transpiration. Maximum transpiration occurs neither when the soil is too dry nor when it is too moist. The wilting coefficient is the percentage of water in the soil (based upon dry weight) when *permanent* wilting takes place, i. e., when the plant is not revived by placing in a saturated atmosphere. When the wilting point is reached, the plant can no longer grow, although the water in the soil may be used to a certain extent to keep the plant from dying by completely drying out. The wilting coefficient is greater than the hygroscopic coefficient, i. e., than the percentage of hygroscopic water in the soil, which means that a plant can never absorb all the capillary water of the soil.

The wilting coefficient has been found by Briggs and Shantz (1912) to vary with the soil rather than with the plant, i. e., the wilting coefficient of a soil is approximately the same no matter what kind of plant may be growing on it, although this applies actually to plants of the same general water needs. Corn cannot draw more water from a soil, in other words, than barley or peas as the accompanying table shows, but if mesophytes (corn) and xerophytes (cacti) are compared, a different story might be told.

Type of soil	*Wilting coefficient (per cent)*
Coarse sand	0.9
Fine sand	2.6
Sandy loam	8.3
Loam	11.0
Clay loam	13.6

Below are wilting coefficients for various plants in sandy loam:

Plant	*Wilting coefficient*
Barley	6.3
Corn	6.5
Oats	5.9
Peas	6.9
Rye	5.9
Sorghum	5.9
Tomatoes	6.9
Vetch	6.1
Wheat	6.3

As stated above, the wilting coefficient is always greater than the hygroscopic coefficient. In fact, the results of Briggs and Shantz have shown that the two coefficients are always related to each other as 100:68, that is, the wilting coefficient is

$$\frac{\text{hygroscopic coefficient}}{0.68}.$$

The *moisture holding capacity* of the soil is the percentage retained against gravity when free drainage is possible. The wilting coefficient equals

$$\frac{\text{the moisture holding capacity—21}}{2.9}.$$

Before wilting, plants may draw excessive amounts of water from the fruits, resulting in the shriveling of the fruit (apples, grapes, and *Citrus*) or causing the physiological disease known as "drought spot" and "bitter pit." The fruit also may be shed early and in an immature condition (fleshy fruits and cotton).

Internal Factors.—By *internal* factors we mean those connected with the plant and which do not lie outside it. One such factor is the age. When the leaves and plant parts are young, transpiration is greatest. Later, as the cutin and suberin form, the epidermal cells are not so permeable, which state connected with the decrease in soil water as the season advances, results in a gradual diminution in transpiration from spring to fall. This may also be explained in part by the accumulation in the leaves of salts, which tends to check transpiration.

Transpiration may be retarded also by various regulatory devices, the most important of which are the stomata. The stomatal apparatus consists of epidermal cells called *guard* cells arranged in pairs on the two sides of an opening. These cells are generally elongated, contain chlorophyll (unlike the other epidermal cells), are fastened firmly to the adjacent cells, and are thicker walled on the side nearest the opening. These characteristics are closely connected with their function. As shown by Fig. 15, which represents an artificial stoma, anything which will increase the pressure inside the guard cells will cause the opening to become larger. As the light falls on the guard cells, sugars are made which increase their osmotic concentration; in many cases this may reach 10–20 atmospheres and be 2–3 times as great as that of the adjacent cells. Water then enters, the cells become turgid, and the opening increases in size, permitting gases to enter to the cells of the mesophyll. This ability to take in water is also influenced by the hydrogen ion concentration which changes as the CO_2 is removed by photosynthesis. The acidity of the guard cell contents is hence a factor in their behavior, as shown by Scarth (1926).

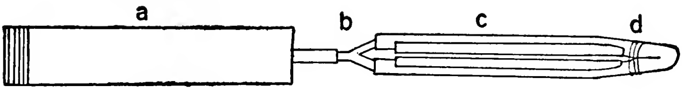

Fig. 15.—An artificial stoma. a, an ordinary air pump; b, glass connection; c, a rubber tube bearing on the inner surface an extra thickness of rubber; d, wire holding the end firmly in position. When air is forced into c, will the opening between the two tubes become larger or smaller?

It was once thought that the stomata had an enormous regulatory effect upon transpiration. They were conceived of as "delicately balanced valves" which adjusted the transpiration of the plant to a very fine degree. Then Lloyd (1908) published some results which indicated that the stomata had practically no regulatory effect. The truth, as shown by Iljin and Loftfield, doubtless lies between these two extremes. It is *light* and not water which determines directly the action of the stomata. Water is conserved at night when photosynthesis cannot go on and the stomata are shut. If there is enough light and water present, they open in the daytime to admit carbon dioxide, but the light is the deciding factor rather than the water supply. This agrees with the conclusion previously reached, viz., that the leaf is constructed to manufacture food, and that transpiration is, for the most part, a necessary evil accompanying photosynthesis. The stomata may not be as efficient as once thought, but they probably help to a certain extent to regulate the water loss. Otherwise their various modifications and their distribution are very difficult to explain. Most of the stomata are found, in general, on the underside of the leaves. Here they can permit the entrance of the carbon dioxide as well as if they were on the upperside but, protected from the sun, the transpiration will be less. The following table from Weiss, Eckerson, and others indicates the distribution per square millimeter of the stomata on the leaves of various plants:

Plant	*Upper surface*	*Under surface*
Acer pseudoplatanus (Norway maple)	0	400
Betula alba (white birch)	0	237
Ficus elastica (rubber plant)	0	145
Juglans nigra (black walnut)	0	461
Morus alba (white mulberry)	0	480
Olea europæa (olive)	0	625
Cucurbita pepo (pumpkin)	28	269
Lycopersicum esculentum (tomato)	12	130
Phaseolus vulgaris (bean)	40	281
Avena sativa (oats)	48	27
Zea mays (corn)	94	158
Triticum sativum (wheat)	33	14
Nymphæa alba (water lily)	460	0
Pinus strobus (white pine)	142	0

Plants growing in dry localities tend to have a reduced number of stomata (grasses and sedges) or to have the stomata sunken in pits (yucca, pine, and carnation). Other means of checking transpiration are by reducing the entire leaf surface. This is seen in plants such as the cactus (*Opuntia*), paloverde (*Parkinsonia*) ocotillo (*Fouquieria*), and in many other desert plants where the leaves have been dropped entirely or in part and a large proportion of the photosynthesis is carried on by the stems. Other plants (cabbage, sugar cane, wheat) prevent transpiration by means of a waxy bloom upon the cuticle, which is thus made impervious to water. Such modifications are commonly associated with an exceptionally thick cuticle as seen in the agave, pine, and yucca.

The mullein (*Verbascum*), olive, silver poplar, and buffalo berry (*Shepherdia*) possess a thick coating of hairs on the under surface which are thought to protect against excessive transpiration, but the work of Sayre (1920) has shed doubt upon their efficacy. The possession of large quantities of water-storage tissue rich in pentosans and other water-attracting materials such as is found in the cacti, *Agave*, *Yucca*, and many desert plants is of advantage, and there can be no doubt that such "adaptations" have had survival value in the formation of a desert flora. In fact, according to Maximov (1923), the transpiration from many xerophytes is commonly high and their real defensive mechanism lies not in checking transpiration but in possessing cell sap of a high osmotic concentration, thus enabling them to recover after wilting.

Ecological Types on Basis of Water Supply.—Water is one of the prime factors in determining the form and distribution of plants. The development of organized life centers about water, which is necessary in fairly large amounts for protoplasm to function at all. Plants growing in marshes, lakes, and places where there is never any danger of a shortage in the water supply are called *hydrophytes.* Those growing under conditions of a moderate water supply are called *mesophytes,* and to this group belong not only most crop plants but most of the land plants.

Plants which grow in regions where water is very scarce are called *xerophytes.* Of such plants there are many different types, but among the better known are the succulents (cacti, *Sedum, Agave,* and aloes), which have a small surface compared with their volume. Water is conserved within the plant and is transpired very slowly because of the thick, waxy cuticle and the water holding capacity of the mucilages and gums contained. But photosynthesis also is checked in such plants, and their growth is extremely slow.

Other xerophytes are the thin-leaved forms such as the sage brush, so familiar on the western deserts of the U. S. A. These plants have a high transpiration, but they are thus able to live in extremely hot localities, and their deep root systems permit them to replenish the water lost. In extremely dry weather, however, water may be still further conserved by the shedding of leaves and branches. The hairy coverings on the leaves have been thought to be a check on evaporation, but, as mentioned above, the efficiency of these hairs has been found to be very low in this respect, and Maximov considers that they function rather to protect the chlorophyll against the bright light and to protect the plant against grazing animals. These plants, unlike the succulents, have a very high osmotic pressure in their cells and can thus draw most of the water from the soil. There are still other types of xerophytes, but this is sufficient to indicate the variation within the group.

QUESTIONS AND PROBLEMS

1. Where in the leaf does the actual loss of water occur?

2. Why does not the air in the intercellular spaces become saturated with water vapor and stop transpiration?

3. Is there any connection between water loss and the amount of water absorbed from the soil? What proportion absorbed is evaporated?

4. What would be the effect, if any, of growing a plant continuously in a saturated atmosphere?

5. Why do florists often water the walls and walks of greenhouses as well as the plants?

6. Why are wilted plants inefficient?

7. Is it possible for a plant to wilt in an abundant water supply? Explain.

8. Why do wilted plants generally revive at night even when there has been no precipitation?

9. Deciduous trees are sometimes called "seasonal xerophytes." Why?

10. If the water requirement of corn is 500 and if a dry corn plant weighs 200 grams, how deep a layer on the surface of a cornfield would the transpired water make if three plants are planted in each hill and the hills are a meter apart?

11. The Roman Campagna was planted with sunflowers to prevent the spread of malaria. Why?

12. In Australia it was once proposed to import "rain trees" (with high guttation) for irrigation purposes. Discuss the feasibility of this method of increasing the water supply.

13. Before a storm the barometer falls. Reconcile this with the relation between pressure and transpiration as discussed in the text.

14. What is xeromorphism?

15. Does the pH of the guard cells increase or decrease during photosynthesis?

16. What may be the relation between evaporation and fruit decline in lemons?

17. Does the rolling of the corn leaf check transpiration? Which way does it roll? Where are the stomata located?

18. Would you expect more or less transpiration on a peat soil than on a rich loam soil?

19. Leaves generally have a higher osmotic pressure than other organs of the plant. Give two possible reasons for this.

20. In general the osmotic pressure of plant cells varies inversely with the humidity. Why?

21. Evergreen shrubs and trees frequently show a higher osmotic pressure in the cells of their leaves in winter than in summer. Explain.

22. Although alfalfa has a high water requirement, it thrives in the plains regions where water is not very plentiful. Explain.

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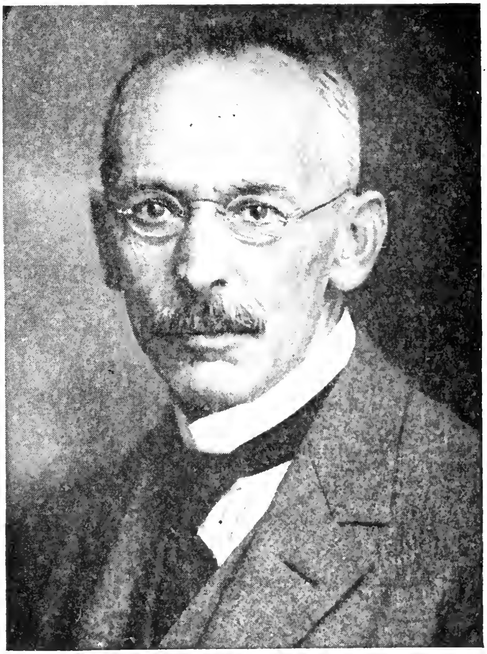

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CHAPTER XXI

THE ASCENT OF SAP

The ascent from earth to heaven is not easy.
—SENECA.

Of the many problems which have long baffled physiologists, that of the ascent of sap has been one of the most perplexing. Everyone is familiar with the fact that water *does* rise from the soil to the tops of the highest trees such as the giant redwoods, which are over 300 feet tall, or to the tops of the eucalyptus trees of Australia, which are even taller, reaching a height of 400 feet and over in exceptional cases. How is the water raised to such great altitudes? What are the forces which are at work to lift such large masses of water such great distances? The problem is very complex because of the variety of forces which come into play, but the chief obstacle to its solution has been our ignorance of the physical and chemical principles involved rather than any inherent mystery in the problem.

Path of Water.—The path of water is from the root to the leaf through the wood or xylem. Water enters the root through the root hairs and traverses the cortex to the central cylinder, where it passes into the vessels and tracheids, going up through them to the leaves. Here the water goes from the vessels and enters the living cells which surround them, where it passes from cell to cell until it is evaporated from a mesophyll cell abutting on an air space. To be sure, some water passes off from the cuticle, and some is used up in the processes which go on within the plant as outlined at the beginning of the last chapter, but the path sketched is that followed by most of the water which enters the plant.

The walls of the endodermis, particularly the radial walls, are partially cutinized, while the tangential walls of the endodermal cells near the end of the xylem regions of the root remain thin as long as the root hairs in that portion of the root are functioning. These *passage cells* thus permit the water which comes in from the cortex to enter the xylem without passing through the phloem of the root; and the sieve tubes of the phloem are thus kept from being diluted by incoming water. In this region the endodermal cells are also especially compact, with no intercellular spaces. These structural characteristics should be kept in mind during the following discussion.

That the water rises in the wood can be shown by two separate lines of experimentation. First, when a tree is girdled, i. e., when a strip of the bark or cortex is removed from around a tree, the leaves do not wilt, and the tree remains active for many days or even weeks. If, on the other hand, by a very careful operation the wood is removed for only a very short distance while the cortex is allowed to remain, the branch will show signs of wilting within a very few minutes if placed in dry air. This shows that the wood is the channel of water ascent, but does not tell us whether the water passes through the lumina (openings) of the wood cells or through their walls. The former would naturally be supposed to be the case, but so great a botanist as Sachs held that the water traversed the walls rather than the lumina. That he was wrong can be demonstrated by plugging up the lumina with wax, paraffin, or gelatin, in which case the plant soon wilts. Furthermore, plants with very large vessels (some of the climbers), which use up large quantities of water, can be placed in water to which drawing ink containing finely divided carbon has been added. In this case, the water can be seen moving into the open ends of the ducts, its path being rendered visible by the opaque carbon particles it carries. Experiments with water containing dyes such as eosin, lead to the same conclusion.

On the whole, then, we can be certain that the transpiration stream rises through the lumina of the wood cells. Inasmuch as the cell walls imbibe water, some water may also rise in them, and it is even possible that some goes up in the cortex; but the water which rises in these places would never keep a plant from wilting even though the roots were abundantly supplied.

Rôle of Osmosis.—The principles of osmosis permit us to understand how the water passes from the root hair to the central cylinder and from the parenchyma cells surrounding the leaf veins to the cells from which the water ultimately evaporates. In the former case, it can be seen that an osmotic gradient between the root hair and the central cylinder will exist which will favor the entrance of water and its osmotic movement towards the central cylinder. As water comes into the root hair the osmotic pressure will be lowered and this cell, A, will have a lower osmotic pressure than cell B (see Fig. 16). That is, there is a higher concentration of water in A than in B and water will go from A to B.

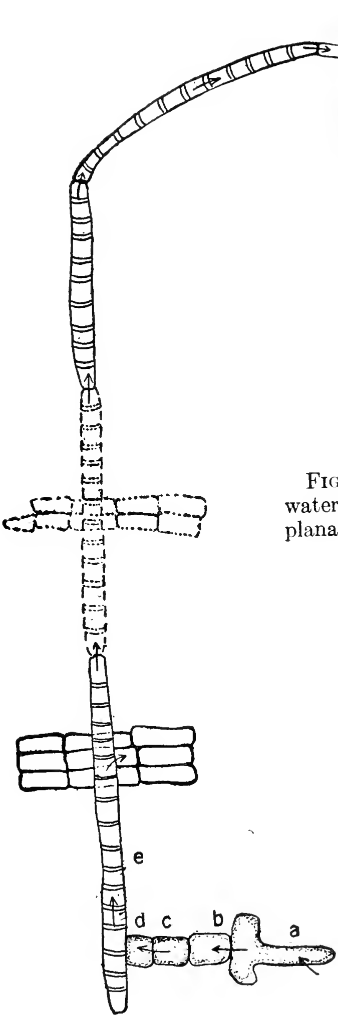

Fig. 16.—Illustrating the path of water through the plant. For explanation see the text.

Cell B will then have a lower osmotic pressure than C and water will pass from B into C. Thus is established the osmotic gradient mentioned above.

Similarly in leaves, as the water evaporates from cell Z, the salts in the cell sap become more concentrated so that Z has a higher osmotic pressure than Y and water will then pass from Y into Z. This increases the osmotic pressure in Y as compared with X, and water will pass from X into Y establishing here another osmotic gradient. Ursprung found that in the ivy leaf the cells nearest the fibrovascular bundles actually had a suction force only about $\frac{1}{3}$ that of the cells farthest away, with a difference of about 20 atmospheres between them. This part of the water movement where living cells are present is, therefore, not difficult to explain. The difficult part has been to find the cause of the movement through the xylem, where the water is passing through the dead cells of the wood.

Among the various factors which have been called in by divers workers to explain the rise of sap through the wood have been capillarity, atmospheric pressure, the osmotic action of living cells in the root and along the stem, and the pull of transpiration assisted by the cohesive forces within the water. Each of these will be discussed in turn.

Capillarity.—It is known that if a fine glass tube is placed in water, the water will rise in the tube to a height which varies with the diameter of the tube. Oil rises in a lamp-wick because of these same surface tension forces. In the soil, the upward movement of water is due to capillarity, and since there are fine tubes in the wood, it was only natural to believe that capillarity might here play an important rôle. The principal objections to this theory are that surface tension forces must have a free surface or meniscus, and in the wood such surfaces do not exist. To be sure, there are free surfaces next the inclosed air bubbles, but for every meniscus concave downwards there is one concave upwards in just the opposite direction to balance it. There is also a free surface in the leaf at the evaporating surface, but this is on the living cells and beyond the region now in question. Another objection is the presence of the numerous cross walls (especially in Gymnosperms which lack vessels), through which the capillary force would have to lift the stream. It is possible that these cross walls would offer no unsurmountable objection (say the friends of capillarity) since they would also imbibe water and thus act along with the capillary tubes of the xylem. If this is the case, they might even be *more* efficient than the vessels because their pores would be still finer!

Other objections are the lack of a free surface below the liquid. Pulling the liquid up from a free vessel would be much easier than pulling it out of living cells below. Furthermore, the cells of the wood, although fine and small, are not small enough to raise the water more than a few feet. If capillarity were the important force, it would be reasonable to expect to find *small* vessels in vines and plants which need much water rapidly. The reverse is found to be the case. Similarly the spring wood, where the most water is carried in a given length of time, contains larger vessels than the fall wood, which is formed in periods of diminished activity. If capillarity were the force involved, the small vessels should be formed in the spring! Plainly to explain the ascent of sap by such forces is out of the question.

Atmospheric Pressure.—Atmospheric pressure has been summoned by some writers to explain the ascent of sap. Their conclusions were reached after finding a negative pressure when a manometer is inserted into a rapidly transpiring tree. This has been interpreted to mean that the intake of water from the soil by the roots is due to the presence of such negative pressures. The leaves, according to this hypothesis, *actively* transpire water which tends to create a vacuum below, with the result that more water is forced in from the outside.

That this argument is fallacious, is easily shown when one recalls that air pressure can operate only when there is a free surface below. Now such a free surface here does not exist. The lower ends of the vessels are separated from the water in the soil by several layers of active living cells in the root. Before atmospheric pressure could act, it would have to force water through all these cells. Furthermore, the negative pressures obtained have never been more than a fraction of an atmosphere, and even though the pressure inside the wood fell to zero so that the weight of the entire atmosphere were available with a free surface to work upon (which has been shown *not* to be the case) the highest the water could be raised under such ideal conditions would be only about thirty-two feet. Atmospheric pressure may help some in small plants and herbs, but can such forces avail to raise water 300–400 feet to the tops of the highest trees?

Pressures less than one atmosphere exist in rapidly transpiring plants as the *result* of the ascent of sap and not as its *cause*. The fact that transpiration from the leaves takes place more rapidly than water can be supplied from below, results in the negative pressures observed. If atmospheric pressure were really efficient, such differences would not exist, and while atmospheric pressure may assist to a slight extent in causing some of the movement of water from cell to cell in the root, it is completely inadequate to furnish an "explanation."

Root Pressure.—Root pressure,—the same force which results in bleeding as described in the last chapter,—has also been invoked to explain the ascent of sap. This force, first noticed by Hales (1735) in a grapevine, is sufficient to burst a bladder tied to the end of the stem and will, under favorable conditions, cause a pressure of 1.5 atmospheres. This pressure, which is the result of turgor in the cells below, might be of some assistance were it present when needed, but unfortunately root pressure is greatest when there is no transpiration and when it is consequently of least use. When the leaves are actively transpiring, little or no root pressure can be detected. Under the conditions that result in bleeding, namely when the top of the plant is removed, it becomes most manifest. This pressure from below could never, therefore, be more than an aid and could not in any sense be the real *cause* of the ascent of sap. Barnes (1910) denied even this and said:

> If a boy could push a wagon while the horse walked, he would be unable to push as soon as the horse's speed exceeded his own. If he clung to the wagon, he would be merely a drag, though if he ran he would be less of a drag than if he made no exertion. The transpiration horse often goes too fast with the water wagon for the root pressure boy to push. Then his grip is broken at once and he is no drag on the load, for root pressure cannot even hold on like the boy and "help" by not being wholly a drag.

This, however, does not seem to be the correct figure. The root-pressure boy does not run along behind the water wagon. Instead he stands by the side of the railroad track while the cars go by and gives each car a little push as it passes. While he does not move the train he may help a little. At any rate he does not hinder its passage or act as a "drag."

A similar pumping action on the part of the ray and other living parenchyma cells scattered along the way between the roots and the leaves has been offered by some as an explanation of the rise of sap. Bose (1922) places these cells in the root and stem next to the endodermis and considers that the xylem acts like a reservoir to meet the demand in times of excessive transpiration. The depletion of this reservoir results in the "negative pressures" observed. Such conceptions, although vague, seem to infer that the living cells at the various levels in the tree act like a system of relay pumps, forcing the water from one level to the next. That living cells are quite unnecessary for the rise of sap, at least in small plants like *Cyperus*, has been shown by J. B. Overton (1911), who killed all the cells of the stem for an inch or more and found that the transpiration stream was not affected by this treatment. That such cells are not *necessary* is hence shown conclusively, but this does not prove that living cells when present may not assist the rise of water. Overton, however (1926), thinks that the living cells in contact with the conducting elements may actually be a hindrance, since they may form tyloses and thus block the vessels.

Cohesion Theory.—The explanation now viewed with the most favor by physiologists is that of Dixon (1909). According to his theory the evaporation of the water from the leaves pulls the water in a continuous column through the tree. The water is like a rope which is pulled up as the upper end vaporizes. This theory consequently rests upon the cohesive forces in water, and the question becomes simply one of whether the water rope, which is 400 feet long in the tallest trees, will hang together without breaking. Physicists have estimated the cohesive force of water to be 10–150 atmospheres, while Dixon asserts that the cohesive force of the sap is at least 100 atmospheres. Ten atmospheres, or the minimum estimated, would be about all that is required and if we add another 10 for internal friction within the cells of the wood, we find that 20 atmospheres would seem to be plenty. With 100 atmospheres present as claimed by Dixon, there seems to be an unusually large safety factor!

Is there an evaporation force which can lift the column of water? is another question which arises. Böhm (1893) in a simple experiment had already shown that the force of evaporation was large. A leafy twig was sealed into the top of a glass tube filled with water and the lower end was allowed to dip into a vessel of mercury. As the water evaporates it is replaced by the water in the tube, and the mercury from below is then drawn up into the tube to replace the water. Böhm found the mercury rose 85–90 cm., which is thus seen to be more than an atmosphere.

Askenasy (1896), in an experiment the importance of which ranks it among the classical experiments of plant physiology, showed that this was a purely physical process by replacing the twig with a dead animal membrane or even by porous gypsum. As the water evaporated from the gypsum, mercury was drawn up from below, in the same manner as in Böhm's experiment, to a height of 80–85 cm., which is also seen to be greater than the height sustained by the atmosphere. Such experiments not only give an idea of the force of evaporation but of the cohesion of the water as well.

The water particles cohere extremely tightly to each other but they also adhere to the walls of the cells in which the water columns are being pulled upwards. This adhesion is so great that when a tree is actively transpiring the walls of the vessels and tracheids are drawn inward slightly as when one draws soda water through a straw. To prevent this, the walls of these cells have spiral and annular thickenings; but that some compression results, nevertheless, may be seen by measuring with a sensitive instrument such as the dendograph designed by MacDougal. This apparatus shows that on bright days when transpiration is active, the diameter of a tree is slightly less than at night when transpiration is much less.

The energy for the transpiration pull is, of course, supplied by the sun. It has been previously shown that only a small amount of the sun's energy is used in photosynthesis, the major part of what is absorbed being used in heating the plant and in evaporating the water. Ganong has calculated that, in a big tree, the work done in one day in lifting the water from the roots to the leaves is equivalent to that done by a man in carrying 500 ten-quart pails full of water up a ten-foot stairs.

Objections to the Theory.—While the cohesion theory is the best and most satisfactory that we have, there are certain objections which have been outstanding. These have largely centered around (1) the gas bubbles in the wood and (2) the cross walls at the ends of the cells.

Will not the gas bubbles break the columns of water? It is a known fact that as the plant transpires rapidly and the negative pressure develops in the stem, the dissolved gases which are in the sap expand and come out of solution just as the bubbles appear in a bottle of soda water when the cap is removed. Why such bubbles do not break the water columns and so render the cohesion theory untenable, is a question which has been much debated, but it seems that because of the irregularities in the shape of the cell wall, caused by the spiral thickenings and other markings, the air bubbles never completely fill the cell and thus break the columns of water. Or if such a rupture *does* happen, then the water is continuous through the cell wall and there is still an unbroken strand of water. According to this conception, therefore, whole sections of the wood might be filled with air without breaking the water rope as a whole. That is, the transpiration stream is not to be considered as a group of separated water columns but as a unit which maintains its unity and integrity throughout the life of the plant. Votchal (1897) even conceived that the air bubbles acted as springs which, by providing increased resiliency, aided rather than impaired the efficiency of the column. MacDougal, Overton, and Smith (1929) concluded that varying gas pressures inside the tree had little effect on the cohesive water system, since changes in gas pressure inside the tree registered on attached gas manometers but did not register on water manometers, indicating that the internal gas system has little or no connection with the water system.

Other objections have been made to the cross walls of the vessels. While these walls are sometimes a meter or more apart, the average length of a vessel is probably less than ten centimeters; and it has been argued that the water could hardly be pulled through these cross walls in the fashion demanded by the hypothesis. Since these walls are full of imbibed water, the molecules of which are in constant touch with one another, they would not seem to offer any insurmountable difficulty to the cohesion theory of the ascent of sap. At all events this theory, although confronted with many facts to explain, has made much progress. It offers a concise, physical explanation of phenomena which were long thought to be mysterious and unexplainable; and while root pressure, capillarity, and atmospheric pressure may play minor rôles in lifting the transpiration stream, the most potent force is the evaporative pull of transpiration, which the cohesion of water permits to be transmitted to the bottom of the plant.

QUESTIONS

1. What and where are the tallest trees? How high are they?
2. In what region of the wood (center or outside) does the water rise?
3. How can a tree be hollow and still live?
4. In tapping trees for maple sugar, how far into the trunk is the tap placed? Why?
5. How many atmospheres must the cohesive force of water equal in order to support the water columns in the trees of question (1)?
6. Define meniscus, cohesion, and adhesion.
7. In the last chapter it was mentioned that leaves commonly have a higher osmotic pressure than stems or roots. In the light of the past three chapters, name two advantages this may be to the plant.
8. Why does air rush into the cut ends of plants unless they are cut under water?
9. Furr reports that in the apple the speed of the flow through the vessels varies with the 4th power of the diameter. Would a dendrograph attached to an apple tree of the same diameter as a pine tree show more or less shrinkage than the pine during active transpiration? Explain.
10. The highest plants in the evolutionary scale have developed vessels instead of tracheids in their water conducting tissue. Name two advantages that vessels possess over tracheids.

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CHAPTER XXII

DIGESTION

> Digestion is the great secret of life.
> —S. SMITH.

Anabolism and Catabolism.—In the preceding chapters, the processes which have been considered were the *constructive* phases of metabolism. These building up processes may all be included in the term *anabolism*, by which is meant those physical and chemical changes within the organism which result in the construction of larger molecules of material from smaller ones. The formation of the carbohydrates, proteins, fats, etc., are all anabolic, but this formation of materials is, in large part, only preparatory to their later use for the release of energy, one of the two main purposes of food. Sugars, fats, and the other foods, which are burned within the plant are not of use until they are so oxidized; hence, after its formation in the leaves or in storage organs, the food must be transported to the place where it is to be burned. The distance which the food must be transported may be very short, but owing to the impermeability of the plasma membrane, large molecules cannot be transported as such. They must first be broken down into smaller particles and put into a soluble form. This putting into solution, which is accompanied by the addition of water, resulting in a product more like water and hence more soluble, is called *digestion*, which is thus seen to be a form of *hydrolysis*. Digestion is one of the processes which result in the formation of smaller molecules from larger; it is therefore a phase of *catabolism* or *destructive metabolism.*

Extra- and Intra-Cellular Digestion.—Digestion is brought about by enzymes which are either produced inside the cell, where they do their work, or else are excreted and do their work outside the cell. The former type of digestion is *intra*cellular and the latter, *extra*cellular. In plants which make their own food and store it up within their own cells, digestion is largely intracellular, while with animals and with plants such as the parasites and saprophytes, which get their food ready-made, digestion is mostly extracellular. The processes are essentially the same but, owing to the source of the food supply, differ in this particular.

In animals, furthermore, this difference in the method of procuring food is accompanied by the presence of a special digestive organ, the alimentary tract, into which digestive enzymes are poured upon the food brought in from outside and in which the foods are made soluble so that they may enter the body of the organism. Plants, on the contrary, have no special digestive organs; but the regions where the food is made and where it is stored must perforce be places where digestion is especially active. In discussing this point, Barnes made the following illuminating statement:

> Misleading comparisons of the leaves to the stomach not rarely occur in primary books, which thus seek to "explain" the work of a leaf. When, as in one notable instance, a leaf is compared to a *kitchen*, where the dilute "soups" coming up from the roots are "boiled down"; later to a *stomach*, where the food is made ready; and finally to the *lungs*, by which the dear little plant breathes, the child would have a truly appalling notion of a leaf were he not usually immune to such bad pedagogy, by reason of his ignorance of at least the stomach and lungs.

As has been mentioned above, the enzymes which bring about digestion are those which add water and which, for this reason, are called the *hydrolyzing* enzymes. Since they are classified on the basis of the material worked upon, we shall here consider them as carbohydrases, glucosidases, esterases, and proteases.

Carbohydrases.—It has been found by Maquenne (1905) and others that starch as stored in plants is not a single substance but is a mixture of amylose and amylopectin. Amylose is soluble in boiling water, turns blue with iodine, and makes up 80–85% of the starch. The amylopectin, which makes up the remaining 15–20%, is not colored blue by iodine and does not dissolve in boiling water. In starch paste, the soluble part comes from the amylose, while the gummy, gelatinous material is the amylopectin. In digestion these are both broken down to dextrin by *amylase* and *amylopectinase* respectively.

The dextrins are intermediate products between starch and sugar. They form gummy solutions and are used in the glue found on postage stamps. The outer crust of bread is rich in dextrins, which have been formed from the starch by the intense heat of baking. These dextrins are formed in three stages, which have been identified by their reactions and have been given appropriate names.

(1) Amylodextrin is nearest starch. It makes up a large percentage of "soluble starch" and is even slightly soluble in cold water. It turns blue with iodine. (2) Erythrodextrin is easily soluble in cold water and with iodine gives a reddish-brown color. (3) Achroödextrin gives no reaction with iodine and has a faintly sweetish taste. Commercial dextrin is a mixture of all of these and gets its sweet taste from this last-named constituent.

The dextrins are then acted upon by *dextrinase* to form maltose, which has been discussed with the carbohydrates (Chap. XII). Maltose is then broken down by means of *maltase* to form glucose.

Diastase is the mixture of carbohydrate enzymes which transforms the starch found in leaves, seeds, and places of especial digestive activity such as tubers and other storage organs, into sugar. That found in leaves is called diastase of translocation, while that in seeds is spoken of as diastase of secretion corresponding to the two starches of the same names, although it should be added that Pringsheim (1926) concluded there was no especial "translocation" starch differing from the normal. While these two enzymes produce the same results, the former has an optimum temperature of about 48° C. and the latter an optimum of about 55° C. Both act best in neutral solutions. Commercial diastase is obtained from germinated barley seeds, while the brand known as *taka* diastase comes from the action of the fungus, *Aspergillus*, on rice or wheat. Since diastase converts starch to hexose sugars, it is really a mixture of amylase, amylopectinase, dextrinase, and maltase.

Invertase (sometimes called sucrase or saccharase) is very commonly distributed in plants. It acts in a neutral or slightly acid medium and breaks down (hydrolyzes) cane sugar into dextrose and levulose. Although cane sugar is soluble, it seems necessary in most cases that it be broken down still further before the molecules can penetrate the protoplasm of the cells. In beetroots where much cane sugar is stored and used, no invertase has been found, which has led some observers to conclude that here the cane sugar must travel as such. The peculiar structure of the beetroot may permit the transport of the larger molecules; but this necessity in general for the further hydrolysis of cane sugar before it can be translocated is an excellent example of the incompleteness of the ordinary definition of digestion, viz., liquefaction. Cane sugar, we have just seen, is soluble and yet it too must be digested.

Inulase hydrolyzes inulin to levulose in the same manner that diastase hydrolyzes starch to glucose. Doubtless here, as in the case of the diastase, there is a series of enzymes which act consecutively.

The pectic enzymes, pectosinase and pectinase, are also important carbohydrases that should not be forgotten in our study of the carbohydrate-splitting enzymes (Chap. XII).

Cytase is an enyzme which acts upon cellulose breaking it down into monosaccharides such as galactose and mannose. Fungi which penetrate the cell wall secrete cytase which permits them to enter. That some soil and water bacteria secrete cytase is evidenced by the gradual disappearance of cellulose (paper) in the soil, and Winogradski has found at least a dozen different kinds of such bacteria. These forms are, for the most part, anaërobic, i. e., they function best in the absence of oxygen, according to Waksman (1926), while fungi play the chief rôle in cellulose decomposition under aërobic conditions. The large quantities of paper which enter the streams and oceans annually in sewage would soon accumulate were it not for the action of these cytase-secreting forms. Cytase is also found in the intestinal tract of herbivorous animals, which are able to use a certain percentage of cellulose as food, so that even straw is to a certain degree digestible.

Gummosis of plants, which was once thought to be due to wounds, is now considered to be due to an overproduction of cytase so that the cell walls are partly digested and broken down into a gummy mass.

These are the principal carbohydrases which are of importance in the digestion and transformation of carbohydrates. It will be recalled that these same enzymes are considered to be responsible for the synthesis of the compounds as well as for their hydrolysis.

Glucosidases.—The glucosidases are those enzymes which break down glucosides into their constituents. One of the best known of these enzymes is *emulsin* found in the almond, peach, plum, cherry, etc., which acts upon the glucoside, amygdalin, and breaks it up into glucose, hydrocyanic acid, and benzaldehyde (Chap. XV):

$$C_{20}H_{27}NO_{11}+2H_2O=2C_6H_{12}O_6+HCN+C_6H_5CHO.$$

In the ordinary almond, emulsin is present and may be extracted for digestive studies, but the glucoside is absent. Because of the HCN formed in this hydrolysis, cattle and sheep which eat wild cherry leaves are commonly poisoned. The same result may be expected when bitter almonds are eaten. Since the HCN is not found until the parts are crushed, it has been concluded that the glucoside and the enzyme are kept apart in the cell by impermeable membranes of some kind, and only when crushed do the two come in contact. In a similar way, the acid of the *Oxalis* leaf and similar leaves with a high acid content may be kept away from the protoplasm.

Salicin from the willow and poplar, and sinigrin from the Cruciferæ are other glucosides whose digestion is discussed in Chapter XV.

Esterases.—Fats cannot be translocated as such but must first be split into acid and glycerin by *esterases*, which include the *lipases* or fat-hydrolyzing enzymes. It will be recalled that the fats are esters of glycerin and fatty acids, so that the term esterase is a more general and hence more appropriate term than lipase, which applies particularly to the fat-splitting enzymes. Lipases have been known in animals for many years, but not until 1890 was their presence observed in plants. At this time Green noted that germinating seeds which were rich in fats or oils acquired an acid reaction. This acid, which results from the breaking down of the fat, catalyzes the enzyme reaction and hence acts like a coenzyme. Tanaka believes the lipase in the seed is in the form of a zymogen and that the acid acts as a transformer. This seems hardly possible, however, since the acid is not absolutely necessary to start the reaction. A small amount of acid added at the start will cause complete hydrolysis to take place within a few hours, while about four days are required if the acid has to be supplied by the hydrolysis itself as the reaction progresses. In castor beans, where lipases have been studied more than in any other seed, lactic acid is the acid produced. Mineral acids may be used to replace the organic acid but much less is required, i. e., the optimum amount is reached much sooner. In Germany, castor beans are used in soap making because of the lipase which they contain, which is used to split the fat preparatory to saponification. It may be added here that Rhine (1926) proposes that fats are often translocated as sugars rather than as fatty acid and glycerin.

Among the other esterases found in plants is chlorophyllase which synthesizes chlorophyll and also hydrolyzes it.

Proteases.—Since protein formation and decomposition proceed to some extent in every cell, the proteases are widely distributed in plants. These enzymes are found in greatest abundance, however, in storage regions which are rich in proteins. The proteins are not stored in as large amounts as the carbohydrates and fats and, for this reason, proteases in quantities large enough to be easily studied are not commonly found.

The proteins, which are broken down to amino acids by stages similar to those found in the hydrolysis of starch, are first acted upon by *pepsinase* to form proteoses or albumoses. These in turn are acted upon by *albumase* to form peptones, which are then hydrolyzed by *peptase* to form the amino acids. The amino acids in some cases (especially through the action of bacteria) are broken down still further by *amidase* into ammonia, skatol, indol, etc. These various steps may be tabulated as follows:

Substrate	*Enzyme*
proteins	pepsinase
proteose or albumose	albumase
peptones	peptase
amino acids	amidase

The different products in the chain of protein hydrolysis to amino acids have different reactions by which the exact stage which the hydrolysis has reached may be told. Some of these differences are given in the following table:

	Proteins	*Proteoses*	*Peptones*	*Amino acids*
Heat effect	Coagulated	Not coagulated	————	————>
With saturated $(NH_4)_2SO_4$	Precipitated	————>	Not precipitated	————>
Biuret reaction	Violet	Reddish brown	————>	No color
Alcohol	Precipitated	————	————>	Not precipitated

Thus if after digestion we find that the product gives a reddish-brown biuret reaction and is precipitated by saturated ammonium sulphate, we may know that digestion has reached the proteose stage only.

In the animal body, pepsin produced by the stomach reacts best in an acid medium and carries the digestion as far as peptones only, showing that it is a mixture of pepsinase and albumase. Trypsin, on the other hand, which is produced in the intestine, works best in an alkaline medium and carries the digestion as far as amino acids. It is thus seen to be a mixture of pepsinase, albumase, and peptase. Ereptases (or erepsins), which are secreted by the small intestine, act on the peptones and polypeptids and break them down to amino acids, but they do not digest the higher proteins. They thus consist mostly of peptases.

The plant proteases, as previously stated, have not been studied so much as those of animals. They are harder to isolate and separate and do not occur so abundantly. Those studied, however, fall into the three classes represented by pepsin, trypsin, and erepsin, and may be called the peptic, tryptic, and ereptic proteases.

Among the pepsins are some which have been found in the insectivorous plants such as the sundew (*Drosera*) and the pitcher plant (*Nepenthes*). The one from *Nepenthes*, like animal pepsin, acts best in an acid medium, but the one from *Drosera* seems to act equally well in slightly alkaline, neutral, or acid media. It should also be noted that these enzymes of the insectivorous plants belong to the class of *extra*cellular enzymes since their action is manifested outside of the cells which secrete them.

The plant trypsins are more common than the pepsins and two, which have been much studied, are especially well known. These are papain from the tropical pawpaw (*Carica*) and bromelin from pineapple (*Ananas*). Papain acts in either an alkaline or acid medium, according to both Vines and Mendel (1902), but better in an acid medium. This led to the hypothesis that this enzyme is perhaps composed of at least two separate enzymes, one of which worked better in acid and the other in alkali, but this has not yet been confirmed. It can be obtained at the drug store as an aid to digestion and is often so used. The proteolytic action is so pronounced that the pawpaw leaves are used by the natives to predigest meats and make them tender. It is said that merely wrapping the meat in crushed leaves results in a marked change within a few hours. Bromelin, like papain, is active in both acid and alkaline media but it works best in a neutral or slightly acid environment.

Plant ereptases are very common in both the lower and higher plants. Not only are they known in bacteria and yeast, but they have been reported also from wheat embryos, green peas, many fruit juices, the various cereals, and from the leaves of lettuce, cabbage, etc. The erepsin of cabbage has been extensively studied by Blood (1910), who found it to be active in acid, neutral, and alkaline conditions.

It has been hard to isolate proteases from resting seeds, and Palladin has stated that no enzymes are present there although they are easily found in germinating seeds. Dean (1905), however, has found enzymes in resting seeds like the bean, and it is reasonable to suppose that seeds such as the bean, pea, and corn, which contain protein reserves, would also contain the enzymes for their digestion unless they are in the form of zymogens and not yet transformed into active enzymes.

In testing for the presence of an enzyme, an extract made of the organ under consideration is placed upon a suitable (carbohydrate, fat, protein, etc.) substrate. In protein studies, gelatin is often used as the substrate or digestive medium.

QUESTIONS

1. Why are parsnips sweeter in the spring than in the previous fall?
2. How is it possible for the delicate filaments of wood-destroying fungi to penetrate the hard wood?
3. Why is digestion a "katabolic" process?
4. Why are substances rich in water more easily digested than those which are not?
5. Give the derivation of the names amylose, amylodextrin, and achroodextrin.

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CHAPTER XXIII

STORAGE AND TRANSLOCATION

> Find where nature moves.
> —Pope.

In the leaf factory, where the initial synthetic products are made, large accumulations of foods would hinder the action and operation of the photosynthetic mechanism. Just as in any ordinary factory the finished products are removed from the vicinity of the machinery at the earliest opportunity to make room for those continually being made, likewise in the leaf factory there must be some arrangement for taking out of the leaf the manufactured materials. These substances are removed to places where they may be immediately used up in the metabolic processes of the plant or, if the plant is making much more food than it is using at the time, the surplus is stored. The requisite conditions for storage are simply that the plant manufactures more of a given material than it can immediately utilize.

Temporary and Storage Products.—Many substances produced within the cell are temporary products, which are very quickly used inside of the cell, or they may be transition products which are rapidly transformed into something else. Some of the simpler carbohydrates, the amino acids, fatty acids, etc., as we have previously seen, are for the most part built up quickly into other compounds; although what is a temporary product in one plant may be a storage product in another.

Regions of Storage.—In the higher plants, owing to their particular methods of growth, large quantities of food may be stored in special organs adapted for the holding of such reserves. These storage organs are generally drawn upon for the growth of the plant at a time when food cannot be manufactured or for the reproduction of the plant. The organs chiefly concerned with the storage of food are:

1. Stems.
    - a. normal stems—cortex, rays, pith, and wood parenchyma.
    - b. specialized stems—tubers, rhizomes, bulbs, and corms.
2. Roots.
3. Seeds.
4. Fruits.

While the ordinary stem is not especially adapted for food storage, certain regions, notably the rays, cortex, and pith, may accumulate large amounts. The rays connect the cortex and the pith so that movement from one region to the other is facilitated. In addition, the wood parenchyma cells scattered through the xylem are commonly found gorged with food.

Of the specialized underground stems, *tubers* and *rhizomes* are of exceptional importance. The former, found on the ends of underground stems as in the potato, store very large quantities of food thus enabling them to serve as organs of vegetative reproduction. The rhizome is a thick, fleshy, underground stem, of which examples are to be found in the iris, fern, sedges, etc. Although the *bulb* as seen in the onion, hyacinth, tulip, etc., is mostly composed of modified *leaves* which act as storage organs, it does contain a small stem and is generally classified as such. The *corm* differs from a bulb in being a solid stem without the leaves and is found in the dasheen (*Colocasia*), Indian turnip (*Arisæma*), *Crocus*, and *Gladiolus*.

Fleshy storage roots are very common. Among them may be mentioned the beet, carrot, parsnip, radish, sweet potato, dahlia, etc. Seeds are also rich in foods and are among the most important of the storage organs.

In the cases above mentioned the food is stored in the organ for the *direct* benefit of the plant which produced it. The food is stored either for the benefit of the plant itself at the beginning of the next growing season, as is generally the case of the food stored in stems and roots, or for the benefit of the young embryo of the next generation, as in the case of seeds. Man, however, as well as other herbivorous animals, interferes and frequently robs the plant for his own uses, as witness the bulk of our food, which comes from roots, stems, and seeds.

Fruits, on the other hand, contain stored food which is only of *indirect* value to the plant which produced it. The survival value of fruits has been the attraction they offer to animals, which thus aid in the scattering of the seeds. Hence fruits, from the point of view of the economy of the plant, belong in an entirely different category from the stems, seeds, and roots.

Relation of Plant Duration to the Storage Organ.—There is a distinct relation between the nature of the plant and the kind of organ in which the food is stored. Annuals, which last only one year and then die, store most of their surplus in the seeds. Thus in the cereals, beans, peas, etc., the seeds are especially rich in stored food. During the one growing season as much nourishment as possible is conducted to the seeds, at the end of which season the parent plant dies leaving the seeds with a good supply of stored food to carry on the life of the species the next year.

Biennials live two years before they produce their seeds and die. Since their life is so short, little or no woody tissue is formed. The first winter they die down to the ground, while the underground parts, i. e., the roots, are the organs which live over the winter. The first season these roots are filled with food which furnishes the materials for the aërial organs when favorable growth conditions arise the second season. For this reason, the majority of the fleshy roots which man uses for food belong to the biennials.

The woody plants which live more than two years may store their food in the stems, and, in general, have no specialized storage organs aside from the fruits already mentioned. To be sure, there is always some food stored in the roots and seeds, but these organs are not especially organized for this function. The plants are generally bulky and the stores of food are not noticeable in any one especial place. The trees and shrubs must store during one season enough food to maintain slow respiration, which continues during the winter, and also enough to produce the young leaves and shoots of the next season. Trees like the peach and almond, and shrubs like *Jasminum* and *Forsythia*, which blossom early in the spring before the leaves have unfolded, must produce these blossoms from food stored the previous season. If this season has been unfavorable for good growth and for the manufacture of food, it is easy to understand why only a small crop of blossoms may be forthcoming.

Reproduction and Storage.—The crop of fruit the previous year may also be a great drain upon the food reserves of the plant. Many varieties of apple do not ripen until after the leaves have fallen or ceased to function, with the result that a part of the food used to produce fruit has come from storage supplies. Since a very large crop one year may seriously affect the size of the crop the following year, this may explain the tendency toward a biennial production of fruit rather than an annual one, even though this may be counterbalanced to a certain extent by fertilizing and careful cultivation, including thinning and pruning.

In some plants it is quite impossible for enough food to be manufactured each year for a good crop of flowers and fruit. The American agave or century plant (*Agave americana*) may spend 20–30 years storing up food materials and water in the thick fleshy leaves before producing any blossoms whatever. Then a thick heavy cluster of flowers is quickly produced, after which the parent plant dies. The desert shrub, *Olneya*, produces seeds only about once every three or four years, but this production seems to depend more upon an adequate supply of stored food, which is impossible under dry conditions, than upon the immediate water supply.

Storage Foods.—The principal storage foods are starch, sugars, proteins, fats and oils, inulin, and reserve cellulose. Starches are by far the most common. In the storage organs the starch is built up from the soluble carbohydrates by means of plastids called leucoplasts. In *Pellionia*, the formation of starch in the leaves and stems by the green chloroplasts can be easily followed with the microscope. The leaf starch is then digested, translocated, and built up again in the form of starch in the storage regions.

The starch occurs in the form of grains which are characteristic for the species and which are used in the detection of adulterants. For further properties of this important group of foods see Chapter XII. Throughout the world plants and organs containing starch are found, many of which, including the cereal grains, buckwheat, beans, potatoes, sweet potatoes, turnips, cassava, sago, tapioca, breadfruit, and bananas, are much used by man for the starch they contain, and serve as the staple articles of diet in the regions where they are found. The potato, which contains 70–80% starch by dry weight, and the cereals, containing 60–80% of starch, are our chief sources of starch in the north temperate zone.

Sugars are found especially in fruits, but are obtained in large quantities from the root of the sugar beet and from the stems of the cane and sugar maple.

Proteins are found among our vegetable foods chiefly in leguminous seeds (peas, beans, and lentils) and among the nuts. The gluten of wheat is protein (Chap. XIV), and some fruits, for instance the banana, contain appreciable amounts of this important reserve, although fruits in general are low in protein content.

The fats and oils are found especially in certain types of seeds. In some members of the lily family, oil replaces starch as the first visible product of photosynthesis, appearing as small drops in the protoplasm. Among the important plants which supply large quantities of commercial oils are the castor bean, coconut, corn, cotton, flax, mustard, olive, poppy, and peanut. In only one of these plants, the olive, is the oil found in a part other than the seed.

Inulin is found as a reserve in the *Dahlia*, artichoke (*Helianthus*), chicory (*Cichorium*) and many of the Compositæ, but it is also present in other families such as the Violaceæ, Iridaceæ, Liliaceæ, Amaryllidaceæ. As mentioned in Chapter XII, it bears the same relation to levulose that starch does to glucose.

Hemicelluloses, which occur in the seeds of the date palm, coffee, peas, and beans, as well as in many other storage regions, have generally been considered of minor importance as reserve foods, but Murneek thinks they play an important part in the storage reserves of fruit trees. While not so readily available as sugars or starches, the hemicelluloses are much more easily hydrolyzed than ordinary cellulose and so may be used in time of seed germination or of leaf and flower formation (fruit trees) when especially heavy demands are made upon the stored reserves.

Time of Translocation.—The foods which are made in the leaves must be moved to the places of storage, and those foods which are stored must later be again translocated to the place where they are ultimately to be used. Storage begins at different seasons of the year, depending upon the plant and the character of the season. Under normal seasonal conditions, the maple begins to build up reserves as early as May, while the oak does not commence till July and the Scotch pine till August or September. Accumulation goes on until the end of the summer in the case of the deciduous trees, or until late in the fall in the evergreens. If the season is late, the beginning of the accumulation of reserves is postponed an equal length of time; but if the season has been favorable, the pith, rays, cortex, and wood parenchyma will contain a large supply of foodstuffs—mostly fats and starch—by the time the leaves fall. The rate of accumulation will depend upon the rate of the manufacture of food and the rate of removal of the translocation products at the storage terminus, just as in an automobile factory the rate of movement of the finished product varies with the rate of manufacture and the rate of removal at the storage or warehouse end.

An annual translocation from the leaves back to the stems occurs in the fall before the leaves die and fall off. Since all material which goes off in the leaves is sheer waste, the plant conserves as much as possible by translocating large amounts back into the stem and the organs below the leaves. Combes and Köhler (1922) have calculated that when leaves die in the fall 45% of the carbohydrates remains in the leaf (cellulose cell walls, etc.), that 35% is burned up in respiration after the chlorophyll has ceased to function, and that 20% is returned to the stem. Much of the nitrogen compounds is likewise saved, and it has been computed that beech leaves send back into the stem 40% of their nitrogen compounds during the yellowing period, while the chestnut may translocate 50% and the horse-chestnut as much as 65%. It should also be added that many of the phosphorus and potassium salts are similarly returned to the stem at this critical period in the life of the leaf. Calcium salts, however, are not translocated to the same extent but remain in the leaves and return to the soil.

There is also a daily periodicity in translocation. During the day the food is manufactured and accumulates in the leaf. At night most of the products of the day's work are removed, and the leaf starts in the following day "with a clean desk." There is probably some removal during the day as well, but the maximum movement occurs between sunset and midnight. Lack of starch in a leaf does not indicate that translocation has occurred but merely that the starch has been transformed (digested). That such translocation does actually take place, however, can be easily demonstrated by comparing the total carbohydrate loss from a leaf attached to the plant with that from one which has been detached. The loss in the former case has been found to be more than five times that in the latter.

Path of Translocation.—The path of translocation has been a subject of much inquiry. In simple plants such as the algæ, floating water plants, etc., no special channels are necessary. Diffusion from cell to cell, according to the ordinary laws of diffusion (Chap. XVIII), is sufficient to account for all the movement necessary. In woody perennials (trees and shrubs) and in the larger herbaceous plants, it is at once evident that the ordinary diffusion processes cannot proceed at a high enough rate to explain the movement observed.

Although Curtis (1920) has questioned the conclusions of previous workers and believes that organic materials are transported upward in the phloem, the *upward* movement from the roots and stems of stored materials is generally conceded to take place in the xylem along with the water and inorganic salts. In the sugar maple it is a familiar practice to tap the xylem (sapwood) and withdraw a portion of its contents, which, with the included sugar, is boiled down into the maple syrup of commerce.

Movement *across* the stem from pith to cortex and intermediary tissues is facilitated by the rays, which consist of living parenchyma cells, which at the end of the growing season are rich in stored foods. These cells are not only connected with the cortex and pith, but are also in communication with the xylem vessels, into which their stored products can be easily transferred.

The chief object of dispute in connection with translocation has been the region of *downward* movement. Sachs (1863) thought that the starch sheath (endodermis) of stems was the region of translocation since the cells there were all found to be filled with this storage product. Because a material is found in a given layer of cells, does not prove that it is carried in that region, however, but may simply mean storage. That this is the case with the starch sheath was later shown by Heine (1888), who removed the endodermis in a set of careful girdling experiments without seriously damaging the other tissues, and found that such treatment neither hindered the development of the plants nor interfered with the storage of starch in the sheath above the wound. This eliminates the endodermis as the region of downward movement.

Although Dixon (1922) and Arndt (1929) have recently revived arguments in favor of the xylem, since the time of Heine workers have fairly well agreed that the region in question is the phloem or inner bark, which is found immediately outside the cambium. That the phloem is the region in which the downward movement takes place, has been concluded from four different lines of evidence, which will be discussed in their relative importance.

1. *Evidence from deduction.* The pith is dead and commonly filled with gases. The wood is occupied with the upward movement of water and materials in solution. The outer bark or cork is also composed mostly of dead cells in which no longitudinal movements have been detected. So with all the rest of the stem eliminated, the phloem is left as the only remaining possibility.

This kind of evidence, while not very scientific, is of interest in connection with the other lines obtained.

2. *Analysis of the phloem contents.* The phloem is extremely rich in food materials which show it to be either a region of conduction or of storage. That it is not a storage region, is shown by the fact that these foods are in solution rather than in a storage form as is the case with the endodermis. An average analysis during the period of active photosynthesis and storage shows the phloem cells to contain about 30% of their dry weight in carbohydrates and 60% in proteins and other nitrogen compounds.

3. *Distribution of the phloem.* The phloem is found in greatest abundance or with larger elements where there is greater need for translocation. In vines with narrow, slender stems and many leaves such as the grape and cucurbits, there is much need for an easy movement of foods between the leaves and the parts below as well as to the ripening fruits. The cucurbits not only have extra large sieve tubes in the phloem, which makes them especially good objects for classroom study, but they have phloem on *both* sides of the xylem (bicolateral bundles). The breadfruit (*Artocarpos*) contains extremely large and numerous phloem elements in the peduncles of its large fruits. Similarly in flower and fruit clusters, where much food must be conducted in from outside, the peduncles may have 50% more phloem than ordinary stems of the same size. In these peduncles the movement is morphologically upward instead of downward and supports the contention of Curtis that upward movement also occurs in the phloem.

4. *The girdling experiments.* In *girdling* or *ringing,* a narrow strip of bark is removed from the stem or branch down as far as the cambium. This strip will include the phloem, which will therefore be interrupted for a distance equaling the width of the removed strip. When the trunk of a tree is girdled, it is observed that the tree ultimately dies but not before the roots become depleted of the food stored in them. Upward conduction of water continues without interruption, but the downward translocation of materials is stopped at the girdle. Food accumulates above the ring, and frequently the stem becomes much swollen at that point. If willows or other similar plants which form roots readily, are girdled and placed in water, roots will form above the girdle only and not below.

While girdling shuts off the transport of food in trees and shrubs, in some plants it seems to have no effect. In squash, for example, removing a ring of tissue down as far as the wood of the bundles does not have the effect which one might expect. The cucurbits, however, have the *bicolateral* bundles mentioned above, with phloem on both sides of the xylem, so that only a part of the phloem is interrupted by the experiment. Likewise girdling is of little effect in monocots, which have the bundles scattered throughout the stem.

Girdling is much practiced in certain regions where it is desired to produce unusually large fruit. A ring or girdle is made some distance from the fruit, permitting the fruiting branch to have several leaves. Then all but one or two fruits are removed from the branch, with the result that all the food produced in the branch goes into these particular fruits. In conducting such experiments, however, one must be careful not to girdle in such a way that the plant will be permanently injured.

The facts that (a) translocation from leaves on one side of a plant tends to result in storage on that same side in many species of plants (Caldwell, 1928), and that (b) chilling a petiole slows up translocation more than it does water transportation (Curtis, 1929) point to the conclusions that the region of translocation (a) is not in the center of the stem and (b) that it is composed of *living* cells.

All of these evidences point to the phloem, therefore, as the region of translocation. Each type of evidence is not conclusive in itself, but the entire array of facts points unmistakably to the phloem as the responsible region.

Structure of Phloem and Its Method of Operation.—The phloem is found to consist of two kinds of cells,—the sieve tubes and companion cells. The former are elongated cells with perforated ends which permit the passage of colloidal materials such as the proteins. The companion cells (Fig. 17), which are smaller in cross section but of the same length, are found in close proximity to the sieve tubes. The sieve tubes lose their own nuclei early in their development, but the protoplasm, which lines their cell walls, remains contiguous with that of the adjacent companion cells and is "under the domination" or "control" of the companion cell nuclei.

Just how the phloem carries on its work and the rôle played by the various elements, are questions still unsettled. The cytoplasm in the sieve tubes by its movements of circulation and rotation probably aids the diffusion processes, which would otherwise go on very slowly. In this process, the nuclei would be of no advantage by their immediate presence but can function equally well from the adjacent companion cells. Downward movement is thus seen to differ from the upward movement in the xylem and to require the coöperation of living cells.

The chief objection to the phloem as the sole carrier of materials downward in plants like the potato and yam (*Dioscorea*), where much translocation must take place, is that it seems hardly big enough. To accomplish the observed results, the downward diffusion must proceed at the rate of about 88 cm. per hour (Mason and Lewin, 1926). Nevertheless, Mason and Maskell (1928) in a series of very careful experiments on cotton found that the mass movements of carbohydrates occurred only in the outer part of the stem, i. e., in the phloem, and that here diffusion of sugar was 40,000 times faster than would occur by *physical* means only. This is to say that down through the protoplasmic cells of the sieve tubes diffusion was 40,000 times faster than a 2% sugar solution would diffuse in water. This is almost as fast as molecules the size of sucrose would diffuse in air! Evidently the living protoplasm of the sieve elements has a way of "getting speed out of" the sugar molecules that we are not yet able to understand. Furthermore, these two workers found (1931) that the inorganic elements (Ca, N, K, P) also travel *downward* in the phloem.

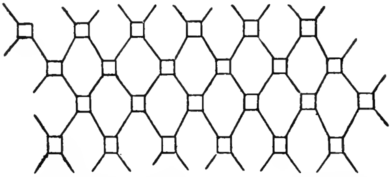

Fig. 17.—A schematic representation of the arrangement of the phloem cells in the corn. The small cells are the companion cells and the large ones are the sieve tubes. In most plants the arrangement is not so regular as in the corn.

Since the phloem is especially rich in proteins (60% of the dry weight in some cases) it has been proposed that a part of the protein synthesis takes place here. Such questions, however, still await their ultimate solution. All that can be said is that the downward translocation of materials in some way takes place in this region, and, as already mentioned, there is evidence to show that some upward movement of stored foods also occurs in the phloem.

Latex.—In some families, translocation and storage take place partly through the latex system. In the Euphorbiaceæ (spurges), Asclepiadaceæ (milkweeds), and the Moraceæ (mulberries and related forms), the latex elements consist of separate cells that may branch and run throughout the entire plant, reaching a length of many inches or even feet; while in the Papaveraceæ (poppies) and many of the Compositæ (lettuce, dandelion, etc.), the latex vessels form a system of branched tubes composed of cells which have fused together, making a complex communicating system between the leaves and the roots. But, whether the cells are separate or fused, they approach very near to the food-manufacturing cells of the mesophyll and are thus well placed to collect the elaborated products of the leaf.

In the latex tubes, the protoplasm forms a very thin layer lining the cells and the vacuole, which contains the watery solution of gums, alkaloids, oils, etc., called latex. Some latex is colorless, but more commonly it is colored as in the bloodroot (*Sanguinaria*) or a milky color as in the milkweed (*Asclepias*), poppy (*Papaver*), and spurge (*Euphorbia*). Commercially latex may be very important as in the poppy, where it is the source of the opium alkaloids, and in the rubber plant.

Whether the latex is a storage reservoir, a waste reservoir, or primarily a conducting system, has not been definitely decided. Some of the products seem to be waste products; others such as the oils and sugars are foods, which in some cases make up as much as 30% of the contained dry matter. In some plants the chief survival value of latex may be in its protective function in that (a) the disagreeable taste and sticky nature of the latex make the plants undesirable forage, or (b) the alkaloids contained render the plants poisonous to insects and higher animals. In the deadly upas tree (*Antiaris toxicaria*) of the tropics, the latex is very poisonous and is used by the natives to poison their arrows. Furthermore, the coagulating properties of the latex of some plants when exposed to the air may be of value in the closing of wounds. In xerophytes, latex may aid in the regulation of the osmotic pressure and help to retain water within the plant. In still other cases, as mentioned above, it may be a food reservoir. In the cow tree of Venezuela, *Brosimum galactodendron*, the latex is palatable and nutritious and is used as food much like ordinary milk. Thus the latex, like the tannins, glucosides, and pigments may have very different functions in different plants. Plants are living organisms, and there is no more reason to believe that those with latex all use it for the same purpose than to think that all men with automobiles have them for the same purpose.

Summary.—In plants where manufacture exceeds consumption, food will be stored. In transporting this food from the regions of manufacture as well as in carrying colloidal materials (proteins) to the regions where foods are used but not made, the phloem cells play the most important part; but they may be supplemented in certain plants by the latex, and in some cases (upward movement) other regions such as the xylem may be the more important.

QUESTIONS

1. Why does a girdled tree ultimately die? Why not immediately?

2. Certain bark diseases, for example, the chestnut bark disease, attack only the bark and yet are fatal. Why?

3. Why do the tissues above swell, when a trunk is bound tightly with a wire?

4. What is "layering"? Why is it successful?

5. Exhibition growers sometimes girdle a branch some distance below the blossom to produce an exceptionally large fruit. What must be present above the girdle besides flowers? Why?

6. Give an example of a product which in one plant is a temporary product and in another is a storage product.

7. At the time of leaf fall, why is a larger percentage of nitrogen saved than of carbohydrates?

8. What is meant by the "morphologically upward" movement of food in fruit peduncles?

9. It is commonly said that for each good apple fruit there should be at least 30 leaves. Why this connection between number of leaves and fruit?

10. Does it seem reasonable that much translocation should occur in the walls of the phloem cells? With this in mind, read critically the articles by Crafts.

11. Name six possible functions of latex.

12. Why should there be any connection between the region of storage and the duration of life of the plant?

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CHAPTER XXIV

RESPIRATION

> And tis my faith that every flower
> Enjoys the air it breathes.
> —WORDSWORTH.

It has been pointed out that the chief use of certain kinds of foods (fats and carbohydrates) is to supply energy for the processes which go on within the organism. During photosynthesis and the various other syntheses which take place within the plant, potential energy is stored in the molecules of material built up. This energy, which is derived from the sun, is of use only when it is changed over into kinetic energy, or the energy of motion. To do work, energy must be supplied, and the only way in which this is done in living organisms is by releasing the potential energy of the food molecules in the katabolic process known as *respiration.*

Respiration and Breathing.—This release of energy goes on in the cells of the body and is not associated with any particular set of organs. Since it is ordinarily accompanied by the addition of oxygen, the gaseous exchange between the lungs of animals and the outer air, by which oxygen is brought in and carbon dioxide released, is sometimes, but erroneously, spoken of as respiration. This is *breathing* and is different from respiration, which takes place in the tissues. Breathing is an *accompaniment* of respiration in animals which have such special organs as the lungs, and is an aid in this exchange of gases, but the real respiration is the oxidative process, which occurs in the cells of the body where the energy is released. Plants consequently never breathe. It has been suggested, in order to avoid confusion, that the term *energesis* be used for the energy-releasing process; but if the word respiration is properly understood and used, the need for a new term disappears.

Respiration in Plants and Animals.—Respiration in plants and animals is essentially the same. In a charged storage battery, the potential energy is of no value until a circuit is completed and the energy is permitted to become kinetic. In a power dam the stored water is of no use until it can fall and by its motion do work.

In a similar way the stored energy of the food of plants and animals becomes available for work in the katabolic process of respiration.

It is a well-known fact that sugar, oils, meat, etc., can be burned by adding oxygen and that, as a result of this combustion, carbon dioxide and also energy in the form of heat are given off. It should not be surprising that similar energy releases can be accomplished in the bodies of organisms. Respiration is essentially the same in its broader outlines as the combustion here described, but the details differ widely from those of ordinary burning. In the latter case, the oxygen seems to unite directly with the substance consumed, the union taking place at a high temperature. In the organism, on the contrary, the union must take place at the comparatively low temperature (generally under 35° C.) of the organism, and this requires the aid of enzymes which can make the reaction proceed rapidly enough to supply all the energy needed even though the temperature is low.

The Gaseous Exchange.—The respiratory equation shows that the food (in this case glucose) is used up with the intake of oxygen, resulting in the ultimate formation of carbon dioxide and water with the release of energy:

$$C_6H_{12}O_6+6O_2=6CO_2+6H_2O \text{ (+3.9 calories of energy per gram).}$$

Like the photosynthetic equation (Chap. VI), this shows nothing of the intermediate steps but only the beginning and the end.

That such a gaseous exchange takes place as is indicated by the above equation can be demonstrated easily by placing germinating seeds in a confined atmosphere and measuring the amount of carbon dioxide and oxygen at the beginning and at the end of the experiment. It will be found that the air, which at the beginning of the experiment was rich in oxygen and able to support combustion, diminishes gradually in this component, and at the end of a few hours will be so much less than at the beginning that a glowing splinter is quickly extinguished. The carbon dioxide, on the other hand, increases in amount. This can be tested by the methods of gas analysis or by passing the air through a solution of barium hydroxide (baryta water). The barium carbonate formed is insoluble and forms a white precipitate, which continues to increase as the carbon dioxide is evolved from the respiring seeds.

Accompaniments of Respiration; Production of Heat.—There are certain accompaniments of respiration which are normally found and which, in some cases, are of much physiological importance. First among these is the production of heat. Not all the energy liberated is used up by the processes of the plant, but a certain amount is freed in the form of heat. Under ordinary conditions this heat is dissipated into the surrounding air so that the plant does not have a higher temperature than the environment, but if it is confined in a calorimeter so that very little heat escapes, it can then be easily measured. The temperature of growing shoots is seldom more than 0.3° C. higher than the surrounding air, but, if plant parts are taken which are extremely active, greater differences can be observed. During germination and during the time of bud unfolding, respiration is especially intense, and at these periods the heat formation can be easily detected. Germinating seeds have been found to give in properly insulated calorimeters a difference in temperature of 20° C. for 100 grams of seed. Seeds used in such experiments must be sterilized or the rise in temperature will be due in large part to the action of bacteria. This temperature increase, to be sure, is the result of respiration but respiration of the bacteria rather than that of the seeds.

Bonnier (1893), who carried on extensive researches upon the production of heat by germinating seeds, found that the amount of heat developed varied with the stage of development of the seedling as indicated in the following table:

Stage of development. Pea seeds	*Gram calories of heat produced per minute*
Soaked seeds	9
Seedlings—roots 5 mm. long	125
Seedlings—roots 55 mm. long	75
Seedlings—stem 20 mm. long	60
Seedlings—cotyledons withering	22
Seedlings—cotyledons fallen	6

As this table shows, the period of greatest heat production is when the roots are about 5 mm. long. Since the evolution of heat is a measure of the excess energy produced over that used, the temperature is not an index to the intensity of the respiration but to the inefficiency of the organism at that particular period. Peirce (1912) found that in germinating peas about 8.5 calories of heat per minute per kilogram were given off during the first day.

In flower clusters differences of 5–10° C. between the temperature of the clusters and the outside air are commonly observed, while in the spadices of some aroids differences of 30° C. have been noted. All this heat eliminated from seeds and unfolding buds comes, in the main, from respiration; but it must not be forgotten that other chemical changes which go on within the plant, such as the enzymic hydrolysis of carbohydrates, as well as certain physical phenomena, such as imbibition, are accompanied by the liberation of heat and may account for a fraction of the heat produced.

Decrease in Dry Weight.—Since respiration results in the oxidation of carbon compounds present in the plant with the elimination of the carbon dioxide produced, a decrease in dry weight is to be anticipated. If a seedling is grown in the dark, the *bulk* of the young seedling may be many times that of the seed, but if all the water is driven off and the *dry weight* alone taken, it will be found that the plant although gaining in bulk has lost appreciably in dry weight. The evaporation of the water produced in the reaction will also cause a loss of weight.

Seeds containing fat or oils do not lose as much dry weight during germination as those which store their reserves in the form of starch and sugar. It has even been stated that flaxseed increased in dry weight, but this has been definitely disproved. Such oily seeds lose so much less dry weight than other seeds because during germination the fat is changed to carbohydrates before it is respired, and in making this change a great deal of oxygen is added to the fatty material. The gain in oxygen through this transformation of fats nearly equals the loss in weight through respiration, with the result that the net balance is almost as great as that of the original material.

While carbon dioxide and water are the principal products of respiration, in some cases, succulents, for instance, respiration is incomplete and organic acids only are formed. These are thought to serve as reserves of carbon dioxide which can later be used in photosynthesis. In the bladder floats of some of the marine algæ (*Nereocystis*), carbon *monoxide* has also been found, but this is a very exceptional respiration product. In this case the gas may reach as high as 12% of the included gases but it is found only when the plant is supplied with oxygen, indicating that it is connected with respiration rather than with other processes like photosynthesis.

Production of Water.—As seen from the respiratory equation, water is one of the products formed. Since water plays such an important rôle in the plant, is found in all parts of it in larger or smaller amounts, and is used in so many different reactions within the organism, it is extremely difficult to determine the exact amount formed by respiration. Liaskovskii (1874) studied the formation of respiratory water in germinating seeds, and Babcock (1912) made extensive studies on germinating seeds as well as on insects like the clothes moth, which lives on dry wool. This insect takes in practically no water and is able to live from the water produced in respiration. In a similar way, the water in desert plants and animals may in part be accounted for. This respiratory water is of higher importance in animals than plants, since the plant is only getting back in respiration the water which it previously had to take in for the manufacture of the carbohydrates. To assume that such water is really being furnished the plant is arguing in a circle.

Activity of Respiration.—The formation of heat, loss of dry weight, and amount of water produced, all vary with the activity of the respiration, which in turn depends upon many factors (to be discussed in the following pages), but to say that respiration is very active is a mild statement. It is commonly thought that respiration in plants is weak compared with that of animals, but, when compared weight for weight, it is found that the difference is not so great as one would expect in light of the greater *apparent* activity of animals. Thus a man at light work produces about 1.2% of his weight in carbon dioxide every twenty-four hours. Horse-chestnut (*Aesculus*) buds have a carbon dioxide output which equals 3% of their dry weight in the same length of time; poppy seedlings 2%; and molds as much as 6%. If heat effects are compared, a kilogram of germinating peas will give off about 25% less heat per minute than a mouse; while if the consumption of oxygen is compared, young wheat leaves are found to use about the same amount of oxygen per hundred grams of weight as a man, unfolding flower buds four times as much, and some bacteria two hundred times as much! All this indicates that respiration is not as slight in plants as is often thought.

The question arises whether respiration from plants in sleeping rooms is sufficient to be dangerous to the occupants of the room. While the amount of respiration is relatively high in proportion to the weight of the plants, it has been computed that for ordinary house plants about 300 sq. m. of leaf surface are required to equal the amount of carbon dioxide given off by a person in the same time. This would lead to the conclusion that plants in a sleeping room are not harmful and that one can continue to sleep in forests without danger of asphyxiation.

Respiration and Photosynthesis.—Respiration is most easily detected in opening flower buds and germinating seeds, not only because the plant is very active at that time, but also because where chlorophyll-bearing tissues are concerned, it is always difficult to separate fully respiration and photosynthesis and to measure the products given off in the one operation which are immediately consumed in the other. Photosynthesis uses carbon dioxide and water giving off oxygen, while in respiration the reverse process takes place. In daylight the photosynthetic activity is so much more vigorous than the respiratory that the latter is completely masked. All the carbon dioxide given off by respiration is used up in photosynthesis and still more is brought in from the outside, with the result that the plant *increases* in dry weight. It is for this reason that one sometimes finds in elementary books and popular papers the statement that animals take in oxygen and give off carbon dioxide in their respiratory processes while plants do just the reverse, taking in carbon dioxide and giving off oxygen. This is, of course, erroneous. Plants respire as well as animals, but in addition they possess the power of photosynthesis which animals lack, so that under the conditions which favor photosynthesis the respiration is hard to detect. Both plants and animals give carbon dioxide to the air by respiration, but only plants have the power to return the carbon dioxide from the inorganic to the organic world.

The comparison between photosynthesis and respiration may be more easily seen if the pertinent contrasting facts are tabulated:

Photosynthesis	*Respiration*
1. Only in green plants.	In all plants and animals.
2. Only in the cells with chlorophyll.	In all living cells.
3. Only in light.	In both light and darkness.
4. Constructs food.	Destroys food.
5. Increases dry weight.	Decreases dry weight.
6. Releases oxygen.	Absorbs oxygen.
7. Requires water and carbon dioxide.	Releases water and carbon dioxide.
8. Changes kinetic energy of light to potential energy of food.	Changes potential energy of food to kinetic energy.

Materials Consumed in Respiration.—It was formerly held that the part of the cell which was respired was the protoplasm itself. Although carbohydrates and fats are the chief energy sources of the plant, the idea was prevalent that the protein was decomposed into a carbon-oxidizable portion and nitrogen. The carbohydrates and fats then united with this nitrogen to produce more protein which was in turn incorporated into the living material of the organism and respired. According to this conception, the supply of non-nitrogenous materials was gradually diminished while the supply of protein material remained unchanged, even though it was continually being oxidized, until the carbohydrate reserves were exhausted. Only when the proteins and protoplasm were no longer capable of regeneration was a decrease in the proteins to be expected. This conception was based largely upon the fact that respiration is especially active in young, growing tissues rich in protein; but it has been found untenable.

It is now held that the carbohydrates and reserves of fats are used up *as such* without first being incorporated into proteins or protoplasm. They are the chief sources of energy, and only when they are exhausted, are the proteins which form the chief source of the protoplasmic materials drawn upon. The proteins form the materials of the engine while the *ternary* compounds are the fuel. As in all engines, there is some wear and tear on the machinery which, in this case, is at the expense of the proteins. Germinating seeds lose their protein stores because the protein is being rapidly transformed into the protoplasm, which is needed in large quantities by the many cells produced at this time of active growth and cell formation. The total amount of nuclear protein, i. e., protein in the indigestible condition as found in the protoplasm, actually increases during the germination of seeds according to Palladin (1899). The amount of respiration may also vary with the protoplasm present because the oxidizing enzymes are produced through the activity of the protoplasm.

The Carbon Dioxide-Oxygen Ratio.—In considering the effects of external and internal conditions upon respiration it is necessary to keep in mind the difference between an effect upon the *rate* of respiration and the effect upon the *carbon dioxide-oxygen ratio* or *respiratory quotient.* As shown by the respiratory equation for carbohydrates, the ratio is ordinarily one, i. e., there is a molecule of carbon dioxide given off for every molecule of oxygen taken in, but this is not always the case, for reasons which will be made clear below.

Conditions Influencing Respiration.—Temperature is one of the chief controlling factors in respiration. Plants in the dormant state give off only small quantities of carbon dioxide and in such circumstances can stand much lower temperatures than when active. Seeds have been placed in liquified gases at a temperature of −250° C. and have survived, showing that respiration must have gone on even slowly at that low temperature. Respiration increases up to about 40° as shown by Fig. 18, prepared from tables by Matthaei (1904). After this temperature has been reached, respiration diminishes slowly until the death of the organism takes place, when it may continue for some time at a fairly rapid rate. The cause for the decrease after 40° is probably some injury to the protoplasm or to the respiratory enzymes. Gerhart (1930) found with strawberries that the temperature coefficient of respiration was 2.5 between 5 and 25° C. These figures agree moderately well with those of other workers on various plant materials.

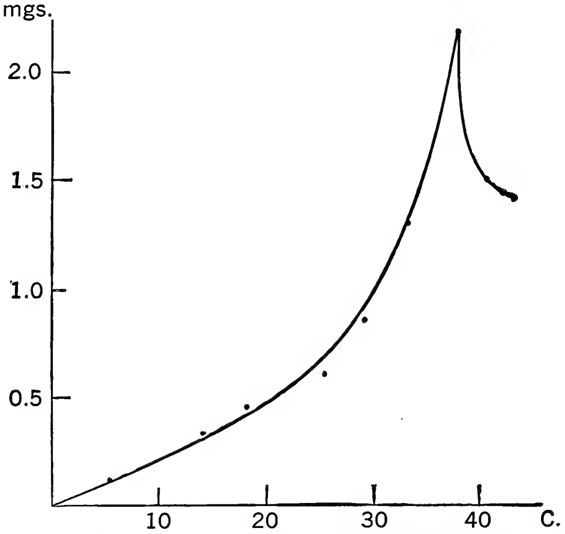

FIG. 18.—Illustrating increase in respiration with temperature. Milligrams of carbon dioxide given off by two grams of cherry laurel leaves per hour at the temperatures cited. (From data of Matthaei.)

Fluctuations in temperature also cause increases in respiration. If a plant is kept for some time at a high temperature and then is quickly transferred into a cooler place, the respiration increases for the first few hours.

The respiratory quotient is at a minimum at about 12° C. according to Purievich (1893) and then increases with both higher and lower temperatures. The following figures derived from studies on the geranium (*Pelargonium zonale*) indicate the kind of results obtained:

Temperature C.	*Respiratory quotient*
4–5	0.75
12–14	0.54
34–35	0.95

At 12° there was about twice as much oxygen taken in as carbon dioxide given off, according to these figures, while at both the higher and lower temperatures the ratio more nearly approaches unity.

Food.—Respiration cannot take place without the presence of respirable matter, the nature of which has been previously discussed. Palladin (1893) found that 100 grams of etiolated bean leaves, containing only a small amount of carbohydrate, produced carbon dioxide at the rate of 90 mg. per hour, but the same leaves when transferred to a sugar solution increased their carbon dioxide output to 148 mg. per hour. No matter how much carbohydrate they contained, the respiration never went beyond this amount, indicating that when the carbohydrate supply ceased to be a limiting factor the respiration could not be increased.

The interdependence of the various factors that affect a given end product may well be illustrated here. We have seen that a decrease in temperature decreases respiration and also that an increase in available food increases respiration. When potato tubers are placed in the cold, as pointed out by Hopkins (1924), this causes the starch to be transformed into sugar, which is more readily burned (respired) than the starch, and respiration increases. Thus we see that the decrease in respiration caused by the drop in temperature is more than counterbalanced by the increase produced by the addition of available (respirable) food materials.

The *nature* of the food respired is also of importance. The carbohydrates and fats are the chief materials respired. Most of the work on respiration has been done with carbohydrates, and concerning the fats very little precise information is available.

To what extent the products of hydrolysis of fat are immediately consumable, is also a matter which remains for further study.

The nature of the food (fat or carbohydrate) which is being used affects decidedly the respiratory quotient. During the respiration of fatty seeds the respiratory quotient is extremely low. Since the fats contain less oxygen than the carbohydrates in proportion to the hydrogen present, they are more efficient energy producers per gram of material and use more oxygen if they are completely oxidized. The equation which represents the complete oxidation of triolein is $C_3H_5O_3(C_{18}H_{33}O)_3+80O_2=57CO_2+52H_2O$, which shows that for every 80 molecules of oxygen taken in only 57 molecules are released in the carbon dioxide. The respiratory quotient is thus $\frac{57}{80}$ or 0.71.

When these fatty seeds ripen, the oil is formed from carbohydrate with the release of oxygen which makes the quotient *greater* than 1 at this season. Thus ripening poppy fruits have been found by Godlewsky (1882) to have a respiratory quotient of 1.5.

Light.—Light increases respiration as observed by Borodin (1876), who exposed to the light leafy twigs which had been kept in darkness. This may be explained on the basis that the light affects the food supply, which is directly related to respiration as noted above. This is supported by the fact that the same wave lengths which increase photosynthesis also increase respiration. Light may have a further influence in that it increases the temperature. Bonnier and Mangin (1884) and Spoehr (1915) find, in addition, a direct light effect which is independent of photosynthesis and heat, since it is observed in heterotrophic plants under constant temperature. The effect is very small, but Spoehr attempted to explain it on the basis of the ionization of the air by light, which might possibly accelerate the oxidative processes. Middleton (1927) found that when barley plants were placed for an hour in air ionized by polonium and then placed in normal air, respiration was as much as 30% higher in the ionized air. Whimster (1927) found with *Pelargonium zonale* an increase of as much as 85% in ionized air; and these effects were found to be due to the ions themselves and not to the ozone produced.

In exceptional cases, e. g., *Spirogyra*, respiration is increased in the dark, owing probably to the fact that nuclear and cell divisions occur mostly in the dark in this plant. It is seen, therefore, that this is no exception to the rule that increased respiration parallels increased cellular activity.

Oxygen and Aëration.—Since the amount of oxygen in the surrounding air is related to the ease with which it is taken up by the respiratory enzymes, the oxygen partial pressure has an influence. The more oxygen the greater the oxidation if oxygen has been a limiting factor. If it is not, as emphasized by Hee (1930), then increasing the oxygen supply does not increase the respiration. In accord with the law of limiting factors, this is what we should expect.

Since roots, like all other living organs, require oxygen for respiration, the importance of good soil aëration is obvious. Clark and Shive (1932) found that a continuous aëration of nutrient solutions containing tomatoes, increased the size and weight of tops and roots 1.5–2.76 times those of nonaërated controls. The poor aëration may harm the plant either (1) by permitting a deficiency in oxygen (the less usual case), or (2) by poisoning the plant with an excess of carbon dioxide. Thus in buckwheat cultures, if nitrogen is passed through the culture solution instead of oxygen, the plants will survive for some time (Free, 1917), but if carbon dioxide is passed through the solution, the plants soon wilt and die. This is of especial importance in the soil, where great quantities of carbon dioxide are being added through the activity of soil organisms. Here this lack of aëration may not only check normal respiration and growth, but it may also render the plant more subject to disease and more susceptible to the attacks of harmful fungi.

Toxins and Anæsthetics.—Various toxic substances, among which may be mentioned alkaloids, glucosides, formalin, alcohols, and anæsthetics such as ether, chloroform, etc., have been studied in their effect upon respiration. In general, the effect of these materials is to cause a stimulation of respiration at the outset followed by a decrease after a short time. As the dose increases, the stimulating effect lasts a progressively shorter time or may be absent entirely, while the decrease in respiration becomes very noticeable as it rapidly falls off to zero.

Concentration of the Nutrient Solution.—According to Palladin (1902) the concentration of the nutrient solution has a marked effect upon the respiration rate. The more concentrated the solution the less the respiration, as the following table shows:

Concentration of medium in per cent	CO_2 *produced per hour in mg.*
0	154.0
15	122.7
25	79.4
50	69.7

Changing the osmotic pressure may have less effect upon the respiration of marine algæ, which live in a medium of high osmotic pressure, than upon that of land plants. Thus the respiration of *Fucus vesiculosus*, which lives between tides and is used to great changes in the osmotic pressure of the environment, is affected very little by changes in osmotic pressure; the respiration of *F. serratus*, which lives in deeper water, is much more sensitive to changes in the osmotic pressure of the surrounding water.

In regard to the respiratory quotient, Purievich (1900) obtained with *Aspergillus* a quotient of about one with a 10% sucrose solution, while on increasing or decreasing the concentration the quotient diminished.

Water.—The influence of the concentration on respiration is directly connected with the effect of water. As might be expected, an increase of water results in an increase of respiration up to a certain optimum. As plants dry out, respiration decreases, with the result that in resting seeds with very little water content, respiration proceeds at a very slow rate. Not all plants have the same water optimum but all are influenced by the amount of water present. The explanation of this is not agreed upon because of the many parts which water plays in plant activities. All processes require an optimum degree of turgor in the cell; and for the formation of the enzymes, as well as for the oxidative process itself, a certain minimum of water is necessary.

Salts, etc.—The action of the various mineral salts on the intensity of respiration varies with the nature of the salt, the concentration, and the other salts present. From what has been said in previous chapters on the effect of concentration and on the balancing function of salts (Chap. XI) this is not surprising. Also a high concentration of salt will tend to lower the supply of available water (Chap. XIX), and part of the salt effect is due to this, but there are many specific effects in addition. For example, phosphates first decrease and then accelerate the respiration of *Elodea* (Lyon, 1924), and nitrogen compounds, especially amino acids, decidedly increase respiration.

Acidity.—The degree of acidity or alkalinity plays an important part in the rate of respiration. This differs for different plants. *Nitrosomonas*, one of the nitrifying bacteria, shows the greatest respiration in a medium which is slightly alkaline; while *Penicillium* shows the greatest rate in a medium distinctly acid. With the seaweed, *Laminaria*, decreasing the pH value from 7.9 to 6.9 (i. e., increasing the acidity) decreased the respiration, while changing from pH 7.9 to 8.75 increased it (Hoffmann, 1929). In the higher plants, the cell sap is generally acid while the protoplasm itself is found to be slightly alkaline. Under normal conditions these environments probably remain fairly constant.

Wounding.—Injuries such as wounding increase the respiration. Richards (1897) has shown that in potato tubers, onions, carrots, etc., there is a pronounced increase in respiration for about twenty-four hours after wounding, followed for four days by a gradual decrease until the normal respiration is again reached. In one experiment 200 g. of potatoes, which gave off 2 mg. of carbon dioxide per hour, were wounded by slicing lengthwise. In twenty-seven hours the potatoes were giving off about 16 mg. of carbon dioxide per hour, or were respiring at eight times their normal rate. Not until five days after the experiment started had the respiration rate fallen to near normal. After the wounding there is also an increase of sugar at the cut surface, so that a part of the increased respiration may be due to the extra sugar rather than to a direct stimulation (i. e., wounding) effect. Also part of the extra respiration in these cases is probably due to the exposing of a greater area, which thus facilitates the entrance of oxygen. This is further indicated by the fact that merely bruising the tuber without breaking the surface does not cause a marked increase in respiration (Lutman, 1926). That respiration varies decidedly with the area exposed is shown also by the fact that small potatoes give a relatively higher respiration than big ones. Large potatoes gave 259 mg. of carbon dioxide per kg. in 24 hours; average sized potatoes, 314 mg.; and small ones, 326 mg. (Hoffmann, 1916).

Stimulation.—Other forms of stimulation besides wounding increase respiration. Pollination has been found by White (1907) to increase the respiration of the carpels as much as five to eight times normal in the case of *Pelargonium*. According to Schley (1920), roots which are stimulated geotropically show an increase in respiration, and he was even able to measure an increase on the convex growing side over the concave side during the time of the geotropic response. That stimulation is accompanied by increased respiration, is also not surprising; wherever there is more activity, more energy is required, with the result that respiration is increased.

Rate of Growth and Maturity.—From what has been said in the previous paragraph, it is to be expected that respiration varies with the growth rate. The curve of growth is an S-shaped curve (Chap. XXVI) which indicates that growth starts out very slowly, rapidly increases during the early stages of development, and then slows down as the plant matures. The consensus of opinion seems to indicate that the respiration curve carried through the life of the plant runs more or less parallel to this curve, with the result that the grand curve of *growth* and the grand curve of *respiration*, as measured both by the amount of oxygen consumed and the amount of carbon dioxide given off, are very similar. Although there are many irregularities (some of which will be discussed later), respiration, in general, increases as the plant continues to grow, until after the grand period of growth; then, as growth slows down, respiration also decreases, although there may be a lag in the respiration curve, as reported by Gustafson for tomatoes. Aging tissues generally show a decrease in respiration as found in sugar cane by Bonazzi (1931) and in many other plants by Hee.

The plant, as a whole, at the time of the maturation of the fruit, has reached the period when respiration is decreasing; but the *fruits*, during maturation, commonly show an *increase* in respiration owing to the increase in the sugar content. Gustafson (1929) reports an increase in the ripening of tomatoes, but attributes it to the decrease in pH value occurring at that time, changes in active acidity being more pronounced in ripening tomato fruits than changes in sugar content. This increase in respiration during the maturation of the fruits Gustafson seemed to consider as exceptional, but in the light of the above discussion, we see that this is what might be expected to occur.

The respiration quotient or coefficient decreases until past middle age as the plant grows; the fraction becomes less than one, which means that the plant takes in more free oxygen than it gives off in the carbon dioxide. The explanation for this is thought to rest upon the fact that cellulose and other products which depend upon the assimilation of large amounts of oxygen are accumulating in the plant.

Amount of Enzymes.—Since respiration is dependent upon the presence of enzymes, their amount is closely correlated with respiratory activity. They are dependent for their formation upon the presence of water and the presence of protoplasm, which explains in part the relation which these two substances bear to respiration. The fact that enzymes act only in a medium of a specific acidity will also explain in part the effect of acids and alkalies on respiration. In fact most of the conditions enumerated above affect respiration either through their influence upon the food supply or through their effect upon the formation and activity of the necessary enzymes.

QUESTIONS

1. Why do plants die when the soil is flooded?
2. What are lenticels?
3. Why are the peduncles and petioles of water lilies hollow?
4. Iljin reports that the first effect of drouth is to *increase* respiration. How may this be explained?
5. Just when in plant metabolism does the potential energy of the food become kinetic?
6. If a plant were grown in air freed of oxygen, would it live longer in the dark or in the light?
7. What does the increase in dry weight in a plant actually measure?
8. Why were the plants of the coal measures preserved?
9. Which weighs more per unit volume,—sound wood or decayed wood? Why? Which gives more heat when burned?
10. Why are flowerpots generally unglazed?
11. Name three advantages gained by plowing.
12. What causes black heart of potatoes?
13. Why do succulents like the agave and cacti find a reserve of carbon dioxide in the form of organic acids of especial survival value?
14. Maquenne and Demoussy reported that they had kept *Aucuba* alive in a vacuum in diffuse light for more than a year. How is this possible?
15. What is phosphorescence and its relation to respiration? Why has it not been discussed more fully in this text, dealing chiefly with the physiology of the Spermatophytes?

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CHAPTER XXV

ANAËROBIC RESPIRATION AND FERMENTATION

> Allay the ferment prevailing in America by removing the obnoxious cause.
>
> —WM. PITT (the elder).

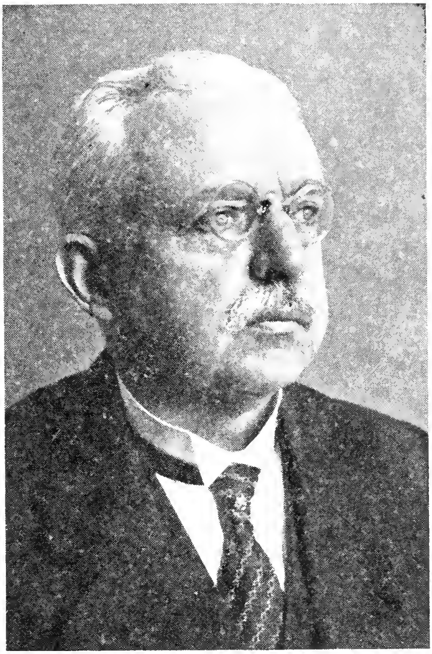
In the previous chapter, respiration has been discussed as a phase of oxidation, but heat and energy can be obtained in other ways aside from oxidizing processes. For example, formic acid was shown by Berthelot (1864) to break down into carbon dioxide and hydrogen with the liberation of heat in the presence of platinum black as a catalyzer:

$$HCOOH > CO_2 + H_2 + \text{heat}.$$

Meyer (1871) showed that yeast could get along without free oxygen, and Pflüger (1875) demonstrated that even frogs could get along for a short time and continue to give off carbon dioxide in an atmosphere devoid of oxygen. In this case, the output of carbon dioxide is only one-third to two-thirds of the normal output, but seedlings of *Vicia faba* will exhale for hours nearly as much carbon dioxide in an atmosphere of pure hydrogen or nitrogen as in an atmosphere of normal air.

Aërobes and Anaërobes.—Some of the bacteria, for example the tetanus bacillus, cannot live in an atmosphere where free oxygen is present. Such forms are called *anaërobes* as opposed to normal *aërobes* which require free oxygen. The tetanus bacillus, which cannot live in the presence of oxygen, is an *obligate* anaërobe, while plants like *Vicia faba*, which *may* get along for a short time in the absence of oxygen, are *facultative* anaërobes. When placed under anaërobic conditions, a normal aërobe stops growing and tends to produce abnormal decomposition products. Seldom, if ever, do anaërobic forms break their food down to carbon dioxide and water; and, for this reason, the respiration is not so efficient as in aërobic respiration, and the total amount of by-products is greater.

That plants are able to respire anaërobically and derive energy



Dr. F. A. F. C. Went, Professor of General Botany, University of Utrecht, Holland. *Tropisms; Growth hormones.*

Dr. W. J. V. Osterhout, Member of the Rockefeller Institute for Medical Research, New York City. *Injury, recovery, and death in relation to conductivity and permeability.*

by processes which do not require the addition of oxygen from the outside, is easily demonstrated by placing seeds of peas, horse beans, etc., in an atmosphere devoid of oxygen. This may be accomplished either by placing in an atmosphere of nitrogen or hydrogen or merely by placing in a vacuum (Fig. 19). Carbon dioxide will continue to be evolved for some time even though no oxygen is present but only so long as there is an abundant food supply. *Vicia faba* leaves were found to give off 782.4 mg. of carbon dioxide when supplied with food and only 256.8 mg. during the same period (30 hours) when not so supplied, in one of Palladin's experiments (1894). In the light of the importance of food in respiration (Chap. XXIV) this is to be anticipated.

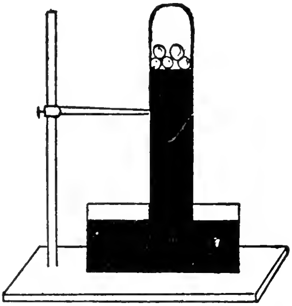
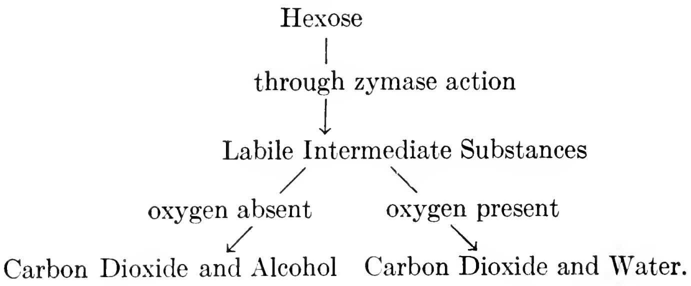


FIG. 19.—Anaërobic respiration. At the beginning of the experiment the test tube is full of mercury and the peas are inserted under the lower rim. At the end of 24 hours the mercury will be pushed out of the tube as shown. Why?

In normal respiration, the food is oxidized (or rather hydrolyzed) and ultimately water and carbon dioxide are produced as end products. In anaërobic respiration, if sugars are present, one of the products commonly formed is alcohol, which can be detected in the anaërobic respiration of seeds, yeast, etc. In the case of the anaërobic respiration of the cultivated mushroom (*Agaricus*), carbon dioxide is found but no alcohol, which agrees with the fact that no sugar is found in this plant.

It was formerly believed that alcohol was formed when sugars were oxidized, whether the process occurred anaërobically or aërobically. If under the former conditions, i. e., in the absence of free oxygen, then the process was considered to stop at that point; but if oxygen was present, then the process was thought to continue until the alcohol was broken down into carbon dioxide and water. This viewpoint has been abandoned for various reasons, but one good argument against this theory is the fact that alcohol is much harder to oxidize than sugar. There is thus no reason to assume that alcohol is an intermediary step in normal respiration. The present tendency is to represent the various steps as follows:



Since pyruvic acid and acetaldehyde have been found in both aërobic and anaërobic respiration, they are doubtless some of the "labile intermediate substances" that are ultimately converted into carbon dioxide and alcohol or water, depending upon the absence or presence of oxygen.

Source of the Energy.—Precisely how the energy is derived in such respiration is not known. It will be seen that even in so-called aërobic respiration, the oxygen does not unite with the food but with the hydrogen produced by its hydrolysis. In this sense, even normal respiration is anaërobic; so that it is not surprising to find that energy is derived from the splitting of the molecules or in other ways which do not require an access of free oxygen. Many plants grow where oxygen is not abundant, as in marshes, flooded soils, standing water, etc. It may be advantageous to them to be able to live without free oxygen, when the power to respire anaërobically would consequently have high survival value in the struggle for existence.

Fermentation.—The term fermentation was originally used to mean the decomposition of organic substances. The breaking down of sugars into alcohol and carbon dioxide, the spoiling of fruit, the souring of milk, the formation of vinegar, the rotting of vegetables, and the development of butyric acid in butter, were all called fermentation. Pasteur was the first to realize that these operations were the result of the growth of microörganisms under conditions (in general) when little oxygen was present, and he therefore regarded fermentation as "life without oxygen." The word came later to mean the decomposition of materials by organisms and was thought to be a property peculiar to life itself, a so-called *vital* process. It is now known that these fermentation processes are all brought about by enzymes, which may be isolated from the organisms and made to do their work *in vitro*. The term fermentation has hence returned to its original meaning as the decomposition of organic materials under anaërobic conditions. There is therefore no fundamental distinction between fermentation and anaërobic respiration, but the tendency still remains to restrict the term to the anaërobic respiration of microörganisms. The various kinds of fermentations are named after the substances produced (alcoholic, lactic, butyric, etc.), and we shall now consider some of the more important of these respirations or fermentations.

Lactic Acid.—The production of lactic acid is seen especially in the souring of milk. Milk sugar (lactose) is used up or respired by various forms of lactic acid bacteria to form lactic acid:

$$C_{12}H_{22}O_{11}+H_2O=4C_3H_6O_3.$$

At least a half dozen different species are able to bring about this type of fermentation. As commonly happens, the products of the fermentation injure the bacteria which produce them, and when about 8% lactic acid is formed, the action is brought to a standstill. If the acid is neutralized with calcium carbonate ($CaCO_3$), fermentation will begin again and, in this way, large amounts of calcium lactate may be formed, from which the free lactic acid may be obtained later. The optimum temperature for this type of respiration is about 38° C. Other sugars, including cane sugar, may be fermented to lactic acid, which is produced along with hydrogen, methane, and nitrogen, depending upon the kind of bacterium involved and the general environmental conditions. According to some investigators, lactic acid may also be produced by roots during anaërobic respiration.

Butyric Acid.—This acid with a very disagreeable odor is developed in rancid butter by various forms of bacteria, chiefly *Clostridium butyricum*, which, in the complete absence of oxygen, breaks lactose or lactic acid down to hydrogen, carbon dioxide, and butyric acid:

$$2C_3H_6O_3=2H_2+2CO_2+C_4H_8O_2.$$

Putrefactions.—In the anaërobic respiration of proteins (*putrefactions*) many disagreeable end products are formed, such as hydrogen sulphide, skatol, indol, etc. The toxins produced by forms like the diphtheria bacterium may also be a kind of fermentation product. Little is known about the details of these processes, but they are undoubtedly advantageous to the bacteria which produce them. The bacteria of decay are also of great economic importance to man, since they perform necessary steps in the organic cycles and thus enable the material of the proteins to be used again by later organisms. Each link in the organic cycle is of as great ultimate importance in the grand scheme of life as any other.

Acetic "Fermentation."—In the formation of acetic acid, ethyl alcohol is oxidized by the acetic acid bacteria to acetic acid and water, as shown by the equation:

$$C_2H_5OH + O_2 = CH_3COOH + H_2O.$$

This is an oxidative process with the addition of free oxygen and is therefore not a fermentation process in the strict sense of the word. Since it is popularly so called, it has been included here, but it should be noted that the formation of acetic acid (aërobically) and the formation of lactic acid (anaërobically) belong in two entirely different categories of reactions.

Alcoholic Fermentation.—Of all the fermentations, this is the one in which most people have been especially interested. From the beginning of written records, alcoholic fermentation has intrigued man and taken much of his attention. Even the passage of laws against alcoholic consumption has not affected the laws of nature and of the fermentation process; it seems much easier to legislate against the activities of human beings than against those of the yeast plant.

The general equation for the fermentation of dextrose by yeast is

$$C_6H_{12}O_6 = 2C_2H_5OH + CO_2 (+\text{energy})$$

but it must be remembered that other organisms beside yeasts bring about the formation of alcohol from sugars. Among these are certain molds and bacteria, and even higher plants may produce alcohol under anaërobic conditions. Other sugars may be fermented and other products developed including succinic acid, amyl alcohol, glycerin, etc., but the chief commercial interest centers in the formation of ethyl alcohol by members of the genus *Saccharomyces* (yeasts) out of hexose sugars.

When glucose is respired or oxidized under aërobic conditions to carbon dioxide and water, one gram molecule (180 grams) produces 709 kg. calories of heat energy (Palladin). When fermented or respired anaërobically, a part of these 709 kg. calories remains in the alcohol. The alcohol derived from a gram molecule of sugar has a total heat of combustion of 652 kg. calories, so that, of the possible 709 calories which can be obtained by complete oxidation under aërobic conditions, only 709–652 or 57 calories are obtained under the conditions of fermentation. Anaërobic respiration is thus seen to be only $\frac{57}{652}$ or less than 9% as efficient as the aërobic method. To supply the same amount of energy the plant must then respire nearly twelve times as much material. The actual difference is even more than that, and all kinds of fermentation require much greater amounts of material than the corresponding complete aërobic oxidations.

It might be expected that the plant would use oxygen if it were available, but such seems not to be the case. In the presence of abundant oxygen, the plant *grows* rapidly, but the amount of *fermentation* is not very much increased. Due to lack of oxidases to combine with atmospheric oxygen, the yeast plant seems unable to use much oxygen even when present, although under aërobic conditions growth and reproduction proceed much more rapidly than in the absence of oxygen. According to the results of Pasteur, the age of the cell plays an important rôle in this connection; young cells are more independent of oxygen than old ones. In the brewing and yeast-growing industries, cultivation is carried on in the presence of oxygen because of its influence on growth and reproduction. The oxygen thus exerts an *indirect* influence upon fermentation.

In the absence of nutrient materials (food) the production of carbon dioxide and alcohol continues for some time. Unlike the ordinary respiration, this *auto*fermentation is accompanied by a loss in dry weight of the yeast plant, since it is now fermenting the food stored up within itself and lacks the opportunity to replace this matter by food from the outside. A similar phenomenon is seen when one compares the changes in dry weight of a plant grown in light, where it can photosynthesize, with those in one grown in the dark, where no food can be made.

The yeasts are of chief importance in the brewing and baking industries. Wild yeasts are found free in nature blowing about in the dust of the air, and it is a matter of common knowledge (these days) that almost any kind of fruit juice which is rich in sugar, will ferment without the *special* addition of yeasts if merely exposed to the air. If the fermentation is produced by molds, generally not more than 5% of alcohol can be obtained, but yeasts tolerate up to 10–15% without becoming poisoned by their own products. After this percentage is reached, the fermentation is stopped. A prohibition law based upon nature would thus abolish all distilled liquors, which are man-made, and permit alcoholic beverages up to the limit found in nature,—up to 15% alcoholic content. This "natural" prohibition law would abolish distilled liquors like whiskey, gin, rum, absinthe, etc., all of which vary in alcoholic content, in the source of the fermentable substrate (corn, barley, fruit, agave juice, etc.), and in the materials added for flavoring (juniper berries, wormwood, etc.).

In the baking industry, the fermentation is carried on for the purpose of obtaining the carbon dioxide and not for the alcohol. In the dough, the bubbles of carbon dioxide gas rise to the surface and thus "lighten" or *leaven* the mixture. During the process of baking, both the carbon dioxide and the alcohol are driven off and at the same time the yeast is killed. On the market two kinds of baking yeasts are available. One is dry and contains much starchy material. It is not so efficient since the proportion of yeast present is smaller, but this type of cake has better "keeping" qualities and does not deteriorate so rapidly. The other type is a moist cake with much water present. It is relatively richer in yeast cells, but must be kept in a cool, moist place. Both types thus have their advantages and disadvantages.

Although oxidases are found to only a very small degree in yeasts, reductases are present and, according to Palladin (1908), play an important part in fermentation. The enzyme of chief importance, however, is *zymase* (Chap. XV). This enzyme may be extracted from the yeast by grinding and crushing and, in fact, is sold upon the market under the name of "zymin." It has an optimum temperature of 30° C. and is killed at 50° if wet and at 85° if dry. It acts only on six carbon sugars, but is found in *Mucor* and *Penicillium* in addition to the yeasts.

Phosphates are necessary for the action of zymase and have been called coenzymes of it. According to the researches of Harden and Young (1910), the process of fermentation takes place in two steps as follows:

$$2C_6H_{12}O_6+2MHPO_4=2CO_2+2C_2H_5OH+2H_2O+C_6H_{10}O_4(MPO_4)_2$$
$$C_6H_{10}O_4(MPO_4)_2+2H_2O=C_6H_{12}O_6+2MHPO_4.$$

The glucose unites with the phosphate forming a hexose-phosphate which in turn is split up, with the addition of water, into glucose and the phosphate. If the phosphates and the zymase are separated by filtration, fermentation is stopped; but when the two are added, the decomposition of the sugar proceeds as before. The phosphate is hence a true coenzyme.

Rôle of Fermentation.—In an elementary text there is not space to go further into the complex matter of fermentation, especially since it concerns the lower plants rather than the seed plants. The precise survival rôle of fermentation is difficult to understand. It seems to be an inefficient substitute for respiration and consequently, under anaërobic conditions, has distinct survival value; in the struggle for existence, plants which *can* live under anaërobic conditions are placed at an advantage. It is hard, however, to see what can be gained by obligate anaërobes which *must* live in the absence of oxygen; and, in those cases in which the organism is poisoned by its own products, fermentation would seem to be a decided disadvantage.

Since, as we shall see in our study of the chemistry of the process, all respiration is to an extent anaërobic, it is possible that anaërobic plants were the first organisms to develop on the surface of the earth, their development occurring at a time when there was little or no free oxygen in the earth's atmosphere. Anaërobic respiration should perhaps, therefore, be considered not in any sense as an *adaptation* but rather as a *stage of evolutionary development.*

Chemistry of Respiration.—Before attempting to discuss this very complex problem, it is necessary to state that we have at the present time no single theory that will explain all the known facts of respiration. Some workers have attempted to form a theory from the phenomena as seen in only plants or animals, without taking into account *all* phases of life; and the tendency has been (as often happens) to form hypotheses in order to explain a particular set of laboratory findings. In the end, many of the difficulties will probably be found to be due to the fact that many of the hypotheses have been formed upon phenomena seen in test tubes, while the organism is a much more complicated piece of apparatus. Thus the study of respiration at present is a jig-saw puzzle. The pieces are here, but they have not yet been put together; and the truth is, we are not absolutely sure that we have all the pieces.

Wieland has called attention to the fact that oxidation probably takes place in a series of stages. Even so simple a reaction as the oxidation of CO to CO_2 takes place in two stages and seems to occur only in the presence of water. The first of these may be represented as: $CO+H_2O=HCOOH$. Then, in the presence of oxygen, the formic acid is oxidized to the carbon dioxide and water: $HCOOH+O=CO_2+H_2O$. The oxygen in this second stage takes up the hydrogen and is thus what is known as a *hydrogen acceptor*. Such an "oxidation" is consequently seen to be more the removal of hydrogen than the addition of oxygen; but, as the chemists have told us for some time, the one is just as much an oxidation as the other.

According to Wieland, oxidations in organisms are generally of this type. The enzymes of oxidation activate the hydrogen in the substance oxidized (respired), and this activated hydrogen is then removed by some sort of hydrogen acceptor. These enzymes are the *reductases*, *dehydrases*, or *oxido-reductases*, so-called because they not only remove the hydrogen but also make it acceptable to the acceptor. The substance respired is the *hydrogen donator*.

Warburg, on the basis of experiments with animal cells and inorganic models, has formulated a theory that emphasizes the physical aspects of the problem. He thinks that respiration is largely a phenomenon of adsorption on the colloid particles of the protoplasm. Using a model of charcoal and a respirable substance composed of cystine and other amino acids, he found that this model would absorb oxygen and give off CO_2, H_2O, NH_3, and SO_3. Iron has been known for some time to be important in animal respiration (Chap. V), and Warburg found that if the charcoal in his model was practically free of iron, it "respired" much more feebly. When traces of iron were added, the gaseous exchange was greatly increased. Furthermore, narcotics (ether and chloroform), poisons, etc., affected the respiration model as they do the organic respiration, the intensity of the effect varying with the degree of adsorption. The one exception was HCN, which depressed "respiration" exceedingly in very small concentrations although it was absorbed only very slightly. This, however, was later explained, when it was shown that HCN readily unites with the iron and removes its influence from the system. Although Warburg tends to minimize the effect of the various enzymes, considered important in most of the other theories, it is admitted that since they are colloidal with enormous surfaces exposed, here also surface phenomena may come into play. The iron is supposed to be either an oxygen carrier or activator, since ordinary molecular oxygen cannot act as a hydrogen acceptor until it has been activated by iron.

Bach and Chodat, the main features of whose theory were enunciated independently by Engler in the same year (1897), have emphasized the catalytic (enzymic) aspects of respiration. According to their scheme of things, for oxidation there must be present oxygenase, a peroxide, and peroxidase. The first named is able to take up oxygen from the air in an active state and thus forms a peroxide. Peroxidases transfer the oxygen from the peroxide to the substance which is to be oxidized.

That two such enzymes are present is deduced from the fact that expressed juices or extracts from some plants will turn a solution of gum guaiac blue when in the presence of air. Other plants will give the blue reaction only when a few drops of hydrogen peroxide are added. Those of the first group seem, therefore, to contain both an oxygenase (able to *get* the oxygen from the air) and a peroxidase (able to *transfer* it to the guaiac); while in the plants of the second group there is a substance present which can transfer the oxygen from the (hydrogen) peroxide to the guaiac, but there is no enzyme present to form the peroxide, which must therefore be added.

Bach and Chodat have applied this to respiration by assuming that respiration takes place in the following steps (to illustrate from the respiration of glucose):

$$C_6H_{12}O_6+6H_2O=6CO_2+12H_2$$
$$\text{A (oxygenase)}+O_2=AO_2\text{(peroxide)}$$
$$AO_2\text{(peroxide)}\rightarrow O+O\text{(active oxygen)}+\text{A(oxygenase)}$$
$$2H_2+O_2=2H_2O.$$

Thus in the plant the sugar of the cell unites with water to form carbon dioxide and hydrogen. The oxygenase takes up oxygen from the air to form the organic peroxide, which is split up by the peroxidase to reform the oxygenase and *active* oxygen, which in turn easily combines at low temperatures with the hydrogen to form water. This explains the visible results as seen in the experiments with the oxidases, and further explains why water is so important in respiratory processes. The food actually combines with water rather than with free oxygen, and oxidation is thus seen to be a kind of hydrolysis.

These same steps are thought to be found in animal respiration as well as in plants. Here the process is made more complicated by the special system (the blood with its red corpuscles) which carries the oxygen from the exterior (lungs) to the tissues. The hemoglobin when united with oxygen becomes red oxyhemoglobin, and when deprived of its oxygen is the bluish hemoglobin of venous blood.

Palladin saw in plant pigments such as some of the glucosides, anthocyans, and the natural plant dyes which vary in their color with the state of oxidation, an apparatus similar to hemoglobin. Many of these, like methylene blue, are colored when oxidized and colorless when reduced. According to this conception, the steps in respiration where these pigments or "respiratory chromogens" (also called phytohematins) are concerned, are as follows:

$$R(\text{pigment})+H_2=RH_2(\text{colorless chromogen})$$
$$12RH_2+6O_2=12H_2O+12R(\text{pigment}).$$

The pigment is thus regenerated and water and carbon dioxide are the ultimate products formed.

If the chromogens are merely "hydrogen acceptors," the entire scheme, which employs the oxidases of Bach and Chodat as well as the respiration pigments of Palladin, may then be represented as follows:

$C_6H_{12}O_6+6H_2O=6CO_2+12H_2$

$12R+12H_2=12RH_2$

$A(\text{oxygenase})+O_2(\text{molecular oxygen from air})=AO_2$ (peroxide)

$AO_2(\text{under influence of peroxidase})\rightarrow A+O+O$ (activated)

$\underline{12RH_2+6O_2=12R+12H_2O}$

Summary: $C_6H_{12}O_6+6O_2=6CO_2+6H_2O.$

Inasmuch as the oxygenase takes the oxygen from the air rather than from the pigment, and since there seems to be no necessity for oxygen carriers in plants as in animals, the advantages of such a scheme are limited to the certain cases where the chromogens are known to occur.

Sufficient has been said, however, to impress the student with the fact that the problems of respiration are far from a unified, coherent solution. It is also hoped that enough has been said to permit those who wish to read the advanced literature on the subject to do so with some degree of knowledge of the terminology there met with and of appreciation of the problems involved.

QUESTIONS

1. What are "vinegar bees"? "California bees"?
2. Should acetic acid bacteria be discussed in this chapter? Why?
3. What is "hard" cider? How can it be kept from turning into vinegar?
4. Fermentation generally develops a high gas pressure while ordinary respiration does not. Why?
5. Give two reasons why manure is added to a hotbed.
6. Discuss the structure and operation of a silo. Upon what physiological principles does it rest?
7. Why do not the contents of a silo decay?
8. If alcohol is made by the yeast plant only from hexoses, why can cane sugar be used?
9. What is glutathion and its relation to respiration?
10. What is catalase and its relation to respiration?
11. Distinguish between *oxygenase* and *oxidase.*

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CHAPTER XXVI

GENERAL CHARACTERISTICS OF GROWTH

> Consider the lilies of the field, how they grow.
> —MATTHEW 6:26.

What Is Growth?—In the previous chapters, those aspects of the life of the plant which deal with nutrition have been studied. The phases of activity concerned with catabolism and anabolism have been the focus of attention. We now turn to the question of growth, which, in fact, cannot be separated from these previous questions except as a pedagogical convenience. The term *growth* involves two distinct ideas which are not always carefully distinguished. First, growth means an increase in size, and, to some writers, this is all that there is in it. Gager states that "the immediate cause of all growth is osmotic pressure," which indicates that he has in mind this purely mechanical increase in size, which is brought about largely by the intake or absorption of water. Thus a flaccid cell placed in water may be said to grow. A dry board which imbibes water is also growing, and this purely mechanical growth may be imitated or reproduced in artificial cells.

It is true that cells will not grow unless they are in a state of turgidity, but other observers have modified this purely mechanical definition of growth by including in it the conception of the formation of new parts or organs. As a plant grows, new tissues and organs are formed, and it is this *formation* and *differentiation* of parts which is the main feature in growth, according to the supporters of this view. In many plants the roots contract during development, pulling the plant more firmly into the ground. *Oxalis* roots may shorten 4–6 cm. In the dandelion (*Taraxacum*) this shortening takes place at the top of the root (morphologically the "stem-root," where these two organs merge into each other in this so-called "stemless" plant) and may equal 20% of the entire length of the "root." Those who emphasize the differentiative and formative phases of growth, consequently, consider that these roots are growing even though they are shortening.

The champions of growth as mere increase in size prefer to distinguish the formation of organs or differentiation as a process entirely apart from growth. Although the two matters may be the result of two distinct processes, increase of size and differentiation are so closely connected that it is practically impossible to separate them fully. In the following pages consequently, both enlargement and differentiation will be considered but, as far as possible, the two ideas will be retained distinct.

Whatever growth may mean it does *not necessarily* include the increase of dry weight. As a grain of corn germinates in the dark, the young plantlet may reach a height of several inches. Much water will be absorbed and the *total* weight of the plant will be many times that of the dry grain. At the same time, the root, stem, and leaves have developed and unfolded. Thus there has been an increase of size and a rapid differentiation of parts, but the plant has *decreased* in dry weight; no food has been manufactured and, at the same time, respiration has been active.

Phases of Growth.—If the cells which make up any organ are studied closely, they will be seen to pass through three stages of growth. The first or *formative* stage consists in the formation of cells by cell division from previously existing ones. In the tips of stems and roots are cells still capable of dividing and of forming new cells. These growing tips are connected by a thin layer of meristematic tissue called the *cambium,* which, like all meristem, is rich in protoplasm, undifferentiated, always young, and capable of producing new cells.

The formative phase passes gradually into the second or *elongative* stage, in which water enters the cells in large quantities; and in the short space of a few hours, the cell may expand to many times its original size. This entrance of water is due in large measure to the pentoses and other "water-attracting" substances inside the cell, many of which are of a colloidal nature as shown by Borowikow (1912), MacDougal (1920), and others. As the water enters, many vacuoles form and gradually coalesce into a few large ones that fill up the center of the cell. Growth is largely due, therefore, not to the formation of much new protoplasm but to the addition of water as the result of either osmosis or imbibition. Although the cells of the root and stem grow in all dimensions, growth is generally greater in length than in the other directions and, for this reason, the period of enlargement is generally spoken of as the period of *elongation.*

After elongation has occurred and the cells have reached their mature size, differentiation begins. Up to this time, all the cells look very much alike. Now the cells which are to be tracheids become thicker walled and assume their characteristic markings; the cells which are to become sieve tubes develop pits in the end walls by the resorption of certain parts of the wall, leaving holes; and cells which are to form the strengthening tissue take on the characteristics of the mature cells. Increase in size is now at an end or takes place very slowly, ultimately stopping completely. It is this phase of growth,—the *maturation* (differentiation) period,—which is considered by some writers as the most important phase and by others hardly to be a phase of "growth" at all.

Grand Period of Growth.—During the period of formation there is very little enlargement, and the same can be said of the period of maturation. The curve of growth is thus an S-shaped curve as seen in Fig. 20. Growth starts in slowly during the formative period, then speeds up enormously during the elongative period, and finally slows down again during maturation. This central period when growth (increase in size) is the most rapid is called the *grand period* of growth, and is found not only in the

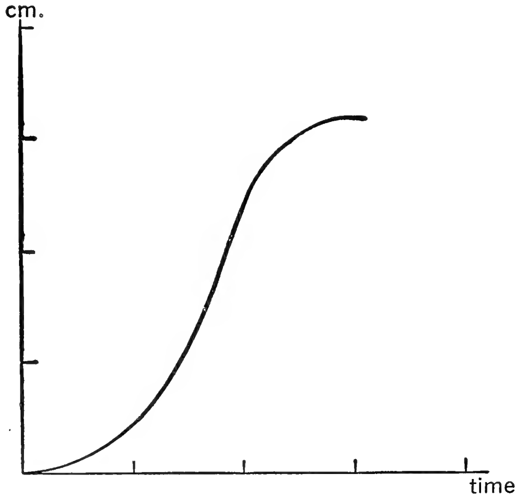

Fig. 20.—The typical S-shaped curve of growth. Growth starts in slowly, then speeds up during the elongation period after which it slows again during maturation.

cells but also in the organs which they compose. In animals these three phases, including the grand period of growth, also occur.

Since the S-curve of growth is the same shape as that of an autocatalytic process, i. e., of a process governed by an enzyme produced as the reaction proceeds, Robertson proposed that growth is governed by an enzyme developed within the cell at the time of cell division. This enzyme diffuses (according to this hypothesis) through the cell and stimulates more growth. But, as the enzyme accumulates in excessive amounts, growth is checked. Although plants do make their own enzymes, this hypothesis is not only inadequate to explain the many variations in the process produced by external factors but it is also unnecessary, since the general S-shape of the growth curve can be more readily explained on a nutritional basis. As the plant starts growth, the reserves from the seed are slowly mobilized; but as germination advances the mobilization of reserves continues at a more rapid pace, and the growth likewise continues at a rapid rate as the leaves unfold and autotrophism starts. Later on in the life of the plant, as sexual maturity is reached, the demands for foods of the flowers, fruit, and seeds shut off the supply to the vegetative organs, and growth decreases. Also, in woody plants, the greater difficulties of transportation attendant upon the increased distance between the roots and leaves tend to cause a slowing down in the growth rate. Thus, while enzymes play a part in all these processes, there is no reason to attribute the various manifestations of growth to a single specific growth enzyme, as was proposed by Robertson.

The absolute rate of growth during the grand period varies in different plants, but a few examples may furnish some conception of the great rates which may be obtained. The stamens of wheat (*Triticum*) at the time of blooming grow for a brief time at the rate of 1.8 mm. a minute, which is about the speed of the minute hand on a watch. If this rate continued for ten hours the stamens would become over a meter long. The leaf sheath of the banana grows at the rate of 1.1 mm. a minute, which is almost as fast, and young bamboo shoots grow at times more than a foot a day. When the century plant (*Agave*) blooms, as it does after 15–25 years, a tall flowering stalk about five meters high and 15 cm. in diameter rises at the rate of about 15 cm. (6 inches) a day. These rates are exceptional, as average growth is about 0.005 mm. per minute, but during the grand period of growth cells commonly increase to 100 times their original size in a few hours, and in the Characeæ they are reported to increase to 2,000 times their original size during the elongative period.

Methods of Measuring.—The rate of growth of plant parts may be measured either directly or indirectly. In the direct method, the size of an organ is measured at known intervals, the increase noted, and the rate computed. The growth may be actually observed by the use of a horizontal microscope. In this method, the microscope is focused on a marked region and the time noted for this region to move a certain distance measurable on the scale of the microscope. The movement will be due to the growth of the parts below and, for this reason, the tip of the plant is the most convenient region upon which to focus.

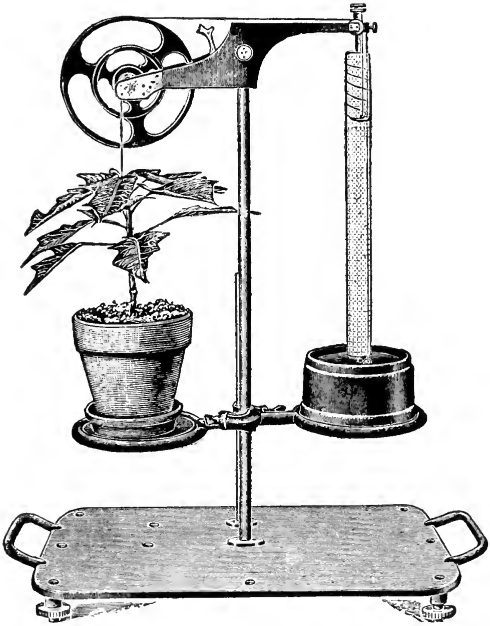

FIG. 21.—The Ganong auxanometer. For explanation of operation see the text. (From Bausch and Lomb Optical Co.)

As an indirect method of measuring growth, an instrument called an *auxanometer* (growth-measurer) is used (Fig. 21). As the plant grows, the wheel rotates and the marker attached to the margin records on the revolving drum the rate of growth, which is magnified by the ratio of the circumference of the rim of the wheel to the circumference of the center, to which the thread from the plant leads. Various types of auxanometers and auxographs have been devised, but one of the most convenient for simple demonstrations is the one devised by Ganong as here figured.

Growth Regions.—All the organs of the plant pass through the phases of growth outlined in the preceding paragraphs. They have their period of formation, their grand period of growth and enlargement, and their period of maturation when they settle down into the business of doing the work for which they are prepared.

In aërial roots the growth region may extend over the last five centimeters or more, but in roots growing in the soil the elongating zone is generally restricted to the region at the very tip. If a root is divided into regions 1 mm. long and marked with India ink, it will be noted that the growth increments are in the last centimeter, the maximum growth being in the region about 3 mm. from the tip. The following figures give the results of a typical experiment with *Vicia faba* roots. The temperature was 20° C., the time 24 hours, and the zones were numbered from the tip upward.

No. of zone	*Growth in mm.*
1	1.4
2	5.9
3	8.1
4	3.7
5	1.4
6	1.1
7	0.4
8	0.3
9	0.2
10	0.1

Furthermore, each zone goes through a similar grand period of growth. The first two days' growth is comparatively slow, reaching a maximum during the next three days, after which it gradually diminishes to zero. The figures given below illustrate the results obtained in experiments of this kind.

Day	*Growth in mm.*
1	2
2	4
3	18
4	18
5	17
6	14
7	7
8	4
9	0

The stem behaves similarly to the root but with the difference that the growth region extends much further back from the tip. The stem is not surrounded by compact resistant soil, which offers an obstacle to growth, with the result that there is not the restraining force imposed upon its growth by the environment as in the case of the root. The region of stem growth varies with the plant, but often extends for several internodes (5–8) back from the tip, covering a region many centimeters long.

The tissues which make up an organ do not all grow at the same rate. As a result of this differential growth, there thus develops within the organ tissue tensions; some tissues are stretched and others are compressed. These tissue tensions may occur either longitudinally or transversely. The former may be easily seen if a stem of dandelion, celery petiole, or other growing stem is cut lengthwise. Immediately after the two halves are separated, the stem will curve so that the outer surface becomes concave. This shows that the pith was compressed and the cortex stretched under the conditions present in the growing stem; but as soon as this tension is released by cutting, the pith then expands while the cortex contracts, with the resultant curvature. If the stem is placed in water, the pith cells take up more water than the cortex so that the expansion of the former becomes still more pronounced and the stem may become much coiled and twisted.

Transverse tensions may be seen best in the cortex of woody plants. Since the wood increases in amount more rapidly than the cortex, which does not split off very rapidly, the wood is compressed by the bark, which is, in turn, stretched by the pressure from within. If a ring of bark is removed as in girdling and is then replaced in its original position, the two ends fail to meet owing to the contraction of the band of bark after its removal.

Leaves grow more evenly than stems or roots throughout their entire surface with a slight increase at the basal region over the apical one. As they unfold, the meristematic region is nearer the base, where the leaf is connected with the stem, and it is in this region consequently where the greatest growth occurs (Fig. 22).

Conditions Influencing Growth.—The factors which affect the growth of plants are either external or internal. Among the internal conditions, *age* and *vitality* are the most important. The connection of the grand period of growth with the age, discussed in the previous paragraphs, is an illustration of the weight of this factor.

Heredity is another factor which cannot be escaped. A sunflower can never be made to grow as large as an oak even with the most favorable of environments. A bacillus may be a micron in diameter, and a redwood (*Sequoia*) may reach the height of over 300 feet. The eucalyptus trees of Australia are even taller than the redwoods, but their diameter and total bulk is much less. Some of the kelps among the brown algæ may reach a length of 600 feet. This shows the great variation in size among the members of the plant kingdom,—differences which are not related to nutrition, light, etc., but are inborn and connected with the past history of the species by bonds so tight that nothing man can do will break them.

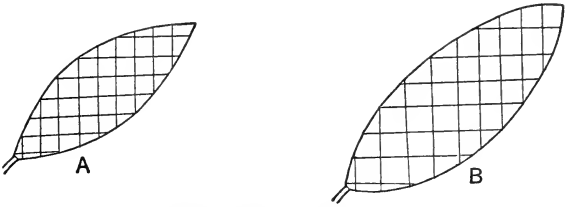

FIG. 22.—Diagram of *Lonicera* leaf showing region of growth. A is a leaf immediately after it is marked and B the same leaf after two days. Note that all parts of the leaf have grown, but the base and apex have grown slightly more than the rest.

A third internal factor closely connected with the external water supply is the *turgidity* of the cell. No cell will reach its maximum size unless there is present enough water to maintain the protoplast firmly pressed against the cell wall. This effect of turgor may be a purely mechanical one but it is none the less important. Unless there is abundant water, the cell will not enlarge or grow.

Periodicity of Growth.—In the temperate climates, growth periods are closely associated with the external environment. In spring when water, temperature, etc., are favorable, growth begins. Later it reaches a maximum and then slows down in the autumn with the shedding of the leaves, as the unfavorable conditions of winter approach. That this periodicity is not purely environmental, however, is shown by the fact that some plants grown in the north and carried to a tropical country where growth conditions are favorable the entire year, still continue to shed their leaves and to retain their regular resting period. Thus the oak, beech, apple, and pear when transported to the subtropical climate of Madeira or to the tropical climate of Ceylon continue to lie dormant during the "winter" months. Other trees have become evergreen in their new environment and have lost the characteristics of their native region very quickly. The peach becomes nearly evergreen the first generation and completely so in the second in Ceylon, where it bears flowers and fruit throughout the year like the oranges in California.

With some plants the periodicity seems to be a question of internal factors and with others it is purely a question of the environment. In the temperate climate, it is a familiar sight to see peach or other fruit trees blooming in the fall if the summer has been a favorable one for food manufacture and when dry weather in the late summer is followed by abundant, warm showers.

Seeds also show a similar resting period. Planted immediately after "ripening" they often fail to grow, but must remain in a dormant condition for some months (Chap. XXXI).

The forcing of buds, bulbs, and other resting organs is a problem whose solution means much to the horticulturist and florist. Aside from providing favorable temperature conditions, much experimentation has been carried on of late with anæsthetics like chloroform, ether, etc. The plants are first exposed to ether vapor for 24–48 hours and then placed under favorable growth conditions. In this way lilacs have been made to bloom in three weeks which otherwise would require double that period. Bulbs and gladiolus corms have been hastened two to three weeks by placing in an atmosphere containing a teaspoonful of ether for every 100 c. c. of air space; and Howard (1910), experimenting with a great variety of trees and shrubs, found that the ether treatment shortened the time required for the opening of the buds about a week or ten days. The effect of the ether is not exactly known, but it probably increases the permeability of the cells, with the result that the food is more readily available.

Hot water has also been used as a method of forcing. The twigs, bulbs, or roots are placed in water at a temperature of 30–35° C. for about ten hours. The effect is similar to that produced by the ether treatment and, because of the practical advantages in application, this method of forcing is to be recommended for ordinary work. Coville, on the other hand, has emphasized the use of *cold* treatment in forcing. He believes that the change from starch to sugar associated with chilling and low temperatures acts as a stimulus and may thus start precocious development.

When one part of a dormant blueberry bush was chilled and the other part kept warm, the chilled portion had leaves and flowers while the other portion was still dormant. It is also his opinion that the forcing effect is associated with an increased permeability of the cells to nutrient materials and to enzymes.

At the Boyce-Thompson Institute these practical phases of physiology are receiving much study, and Denny (1926) has shown that potato tubers can be forced best by ethylene chlorhydrin, of the 224 chemicals tested. Tubers treated with the vapors of this compound produced vines two feet high with young tubers 1 cm. in diameter before the untreated plants appeared above the ground! Shrubs (lilac, *Deutzia*, and *Azalea*) and trees (*Prunus* and crabapple) treated with the vapors of this compound or of ethylene dichloride for 24–48 hours were in blossom 2–8 weeks ahead of controls. It was also possible to force one bud and let the opposite one lie dormant, showing that dormancy is a purely local issue and is not related to the general reserves and conducting tissue of the adjacent stems.

Similarly, ethylene in very low concentrations (1 part in 4,000–10,000) has been used to ripen oranges, bananas, lemons, and tomatoes. Most of these fruits are nearly ripe *internally*, so that the chief effect (but not all) seems to be connected with the development of the pigment characteristic of the ripe fruit. Since ethylene is used also in bleaching celery, evidently its principal action here is to aid the breakdown of chlorophyll preparatory to the formation of the yellow or red pigment of the "ripe" fruit. This "ripening" process may take only a few hours and thus shorten by days the natural processes.

Other methods used (with less success) to hasten plant development and growth are the injection of K, Mn, Mg, and Fe salts (Popoff); artificial light (Niethammer); and wounding (in the case of bulbs). This last method is thought to hasten the increase of water, but all the substances used in forcing, as mentioned above, probably increase the permeability of the cells and may also favor enzymatic changes. These, however, like most vague suggestions, are only another way of saying that we really know practically nothing about the actual *modus operandi.*

QUESTIONS AND PROBLEMS

1. How does the growth of plants differ from that of animals?
2. When is the best season for making whistles from willow and hickory wood? Why?
3. If a nail is driven into a tree five feet from the ground in 1926, how high will the nail be from the ground in 1936 if the tree grows six inches in length each year?
4. Why does the thickness of the cortex of a tree never reach that of the xylem?
5. All the wood of the stem which is produced in any one year produces what type of geometrical figure?
6. What are dandelion "curls" and how are they made? Explain.

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CHAPTER XXVII

SPECIFIC GROWTH FACTORS

> When ye hae naething else to do, ye may be . . . sticking in a tree; it will be growing, Jock, when ye're sleeping.
>
> —SCOTT.

The Growth Effect.—A factor which influences growth may do so in one of three ways. (1) The *direction* of growth may be changed so that movement results as when a plant bends towards the light (Chap. XXIX). (2) A factor may influence the *amount* of growth and (3) it may also affect the *nature of the organs produced.* This latter is commonly called the *morphogenic* effect. The direction of growth will be discussed under "movement," but the effect of *external* factors upon the amount of growth and the organs produced will now be treated in some detail.

Heat.—For growth a minimum temperature is necessary. For most plants 5° C. is the minimum, 30° the optimum, and 45° the maximum. *Opuntia* will grow at 58° C. (MacDougal), which is about the high temperature record for growth among seed plants. As samples of various plants the following figures are included, which show that the optimum is much nearer the maximum than the minimum:

Plant	*Minimum*	*Optimum*	*Maximum*
Wheat	5	30	35
Peas	5	30	35
Corn	10	40	50
Melons	15	35	50

Wheat, as these figures show, can be grown at a much higher latitude than corn and melons, which require rather high minimum temperatures. There is a great variation in plants in this respect. In tropical plants 10–15° is a minimum, in the temperate zone 5–10° is generally the minimum, while in the arctic regions plants grow well at a temperature of 5–10°, the minimum being close to the freezing point. At the other extreme are the algæ found in hot springs, where they thrive at 80° C. and fail to reproduce if the temperature falls below 40°.

When dry, plants can endure much greater extremes than when the protoplasm is rich in water. Thus seeds of the sunflower (*Helianthus annuus*) have withstood heating for a short time to 140° C. or even to 145° if heated gradually; and dry spores and some seeds can endure 125° C. for five minutes and over two weeks in liquid oxygen at −210°, making a total range of more than 300°. Becquerel even reports (1925) that the germination of wheat, mustard, and lucerne seed was unimpaired after these seeds had been for over ten hours in liquid helium (−269° C.)!

In order to bring about a definite degree of development, to cause the ripening of fruits, for example, a certain amount of heat must be supplied. Various investigators have tried to show that the plants of the same species require a definite number of heat units to reach the same state of development; and while it is true that plants grown at the northern and southern limits of their range require a minimum number of heat units, there is much variation, and in estimating the number of units necessary for the development of plants, one must consider not only the external conditions but the internal life of the plant. The resting period may continue in spite of external conditions favorable to growth, so that it is impossible to prescribe a certain number of heat units and always expect to get the same results.

Excessive heat promotes chemical changes which are helpful in development, but it may also promote changes which are harmful. Constantly increasing the temperature is beneficial only up to a certain point, when the harmful effects more than counterbalance the good. This results in the optimum that has already been observed. The damage done by high temperatures (as mentioned above) depends to a large extent upon the amount of water present, and for this reason dry organs can withstand much higher temperatures than those actively growing and rich in water. Wheat, for example, when the water content was 10%, was killed at 78° and corn at 68° C. (Robbins and Petsch, 1932); but when the water content reached 30%, the wheat died at about 53° and corn at about 55°. The temperature coefficient of killing by heat is of the same order as that for the denaturation of proteins (Collander, 1924), which indicates that this is the chief source of danger from high temperatures. This is supported by the fact that guard cells of *Zebrina* and *Rumex* are killed in water at 62° in about one minute if the stomata are closed but not if they are open. The closed stomata are more acid, and this may be associated with the ease of heat coagulation (Weber, 1926).

The effect of cold seems to be somewhat different from that produced by heat. As the plant cools down to near the freezing point (0° C.), the water which is in the cell walls and the intercellular spaces freezes first because of its lower osmotic pressure. In this way water is withdrawn from the cell and the freezing point of the cells becomes lower and lower. It has been estimated that ice crystals will not form in the cells themselves until a temperature of −20° C. is reached. It is for this reason that the degree of injury by cold weather varies with the water content of the cells; juicy, succulent cells are much more easily injured by a drop in temperature than cells with a low water content. A part of the injury is mechanical, caused by the lacerating of the tissue by the ice crystals whether within or without the cells. It may also happen that certain harmful physical changes, similar to the coagulation produced by heat, may result. As the osmotic concentration of the cell sap becomes progressively higher, the time may come when the proteins are "salted out" or precipitated by the excessively high salt content of the remaining liquid.

Whatever the injurious effect of freezing may be, it is certain that it varies with the amount of water present and that the hardiness problem is very closely connected with the water supply. Illustrations of this point have been multiplied the past few years to the place where there can be no further doubt about it: tissues with a high water content and a low concentration of osmotically active substances are much less hardy and resistant to cold weather than tissues with a low water content and a high concentration of sugars, salts, etc. Wheat grains frozen when their water content was 69.4% showed only 1% germination, while those frozen when the water content was only 45% showed 84% germination (Whitcomb and Sharp, 1925). The specific gravity of unfrozen *Citrus* fruits is higher than that of frozen ones after they have been subjected to freezing temperatures, and those leaves that have a lower osmotic concentration in their cell sap freeze at a higher temperature (Haas, 1928). Peach bud hardiness varies in different varieties inversely with the amount of water per unit of dry matter (Johnston, 1923). In the cells of *Picea canadensis* leaves there is a rapid fall in osmotic pressure from over 20 atmospheres to 16–17 atmospheres as the cells pass from the winter to the summer condition, accompanied by a reorganization of the food reserves in the cells (Lewis and Tuttle, 1923). Hardy varieties of wheat are those with a low moisture content and high osmotic pressures (Martin, 1927); and the same was found to hold for winter rye (Tysdale and Salmon, 1926). These random samples from the recent literature could be duplicated many times. That the effect is related to the precipitation of the proteins is indicated by the work of Newton and Brown (1931), who found that sucrose added to the pressed juice of winter wheat protected it against precipitation of the proteins contained up to 8% concentration, a value obtained by hardy varieties in the field; but just how the sugar protects is still somewhat obscure.

The morphogenic effect of temperature is seen when plants which are grown ordinarily at medium temperatures are transferred to mountain tops where the temperature is much lower, especially at night. To determine just how much of the change in form is due to temperature, the conditions have been simulated in the laboratory by surrounding the plant at night with ice and then exposing it during the day to ordinary temperatures. Plants grown under such conditions have the characters so typical of Alpine forms,—limited growth, tough leaves, short internodes, and early flowering season.

An interesting morphogenic effect of heat is seen in the case of cabbage, which, when grown continuously at 70° F. does not behave like a normal biennial but continues to grow vegetatively, reaching a height of over 7 ft. during the second year and having a very different appearance from that of cabbages grown in their natural environment (Miller, 1928). Barley, when grown at 20° C. is large, weak, and sprawling with no flowering shoots (Walster, 1920), while at 15° growth is normal. The acetic acid bacterium, *B. pasteurianum*, when cultivated at ordinary temperatures (30–35° C.) forms chains of short rods, but when grown at 40° C. the cells elongate to form slender filaments which are 100–150 times as long as the original cells. When returned to the lower temperature, the normal form is resumed.

Light.—Although light is absolutely essential to growth (since without it photosynthesis stops), it will seem paradoxical that light, especially strong light, really hinders growth. This checking action of light on growth is a complex matter, not entirely resolved. In the dark, cell division occurs better than in the light

(Brotherton and Bartless, 1918), with the result that in the light growth is due mostly to an increase in *size* of cells rather than in their *number*. Also light causes the stomata to open and favors increased transpiration, thus decreasing turgor, one of the essentials for good growth.

Plants grown in the dark,—so-called *etiolated* forms,—are extremely thin and spindling. They grow rapidly until all the food stored in the seed is consumed, making a really remarkable growth, considering the small amount of dry matter which they have at their disposal. Certain observers have interpreted this rapid, spindling growth as an adaptation. To them, the plant is *searching* for light and, if it grows tall and long, it will have a better chance to *find* it; but it is not necessary to inject such ideas of purposefulness into the explanation. The reason for the excessive growth may be found not in the presence of darkness but rather in the absence of the checking factors accompanying light.

It is a matter of common observation that corn and most plants grow best at night, although the change in temperature and humidity also play an important part in the increased growth. Forest leaves are larger in the shade; and cacti in the bright light are smaller and stockier than those grown in the shade. In the growth of many garden vegetables such as lettuce and cauliflowers, the "half-shade" system of propagation is used, in which the growing plants are cultivated in the shade of cheesecloth placed at a suitable distance from the ground. This cuts down the light, with the result that the plants are more succulent, more tender, with milder odors and flavors, and thus more desirable in a fancy market.

Tobacco is also commonly grown under a half-shade system. When the light is thus reduced 20–40%, a much higher grade of leaf is produced, about twice normal size, and with a much finer texture.

While all these effects are also connected with changes in transpiration as well as with the amount of moisture in the soil, the temperature, and the biological relations with other organisms (such as the fungi) of the soil, there can be no doubt that a part of the effect at least is due to light. That this is true, has been corroborated by laboratory experiments in which these other factors were kept constant.

Strong light, especially that from the blue end of the spectrum, has a very pronounced chemical or "actinic" action, of which the germicidal effect of light is a familiar example. Strong light also inhibits the action of certain enzymes, notably diastase, and this may account for the fact that translocation and growth occur largely at night even though the food is manufactured in the daytime. Although the light of short wave lengths, i. e., the blue-violet, checks growth, normal growth does not occur in the absence of these rays from the spectrum, as shown by Shirley (1929).

Polarized light apparently favors growth processes as shown by Macht (1926–1929) and others. Macht found that under polarized light the seeds of *Lupinus albus* germinated more rapidly and the seedlings grew with improved vigor and at a higher rate than in normal light. Some workers have attributed this favorable effect to an action on photosynthesis. Macht finds that also the processes of hydrolysis are favored, so that polarized light seems to improve the general metabolism. Since a large part of the rays of moonlight are polarized, this has been offered as an explanation of the improved growth observed in many plants on moonlight nights, but it is doubtful whether this explains the improved tonus of college students under this environment.

To bring about development and reproduction each species of plant requires a certain number of light units. To a limited extent, it is not important whether a dim light is supplied over a long period of time or a bright light over a short period; the end result is the same if there is an abundance of carbon dioxide present and if light is the limiting factor. Bailey (1893) found that periods of darkness are not necessary, and, by means of artificial light, hastened the growth of lettuce by two weeks. Incandescent lights are better than mercury vapor lights because of the greater amount of red light which they contain. More recently Garner and Allard (1920) by extensive studies have found that each species requires these units to be given in a particular fashion, with the proper alternation of light and darkness. If this natural rhythm of illumination is changed, the tendency is to change the time of flowering and reproduction, so that it is possible to control the reproduction of the plant as well as the vegetative growth by regulating the light (Chap. XXXI). Furthermore they have shown that the effect of the length of day upon growth is not simply a matter of total energy. Two short periods do not produce the same effect as one long one. Shortening the light period much below 12 hours decreased growth, and with light alternations of 1 minute growth was at a minimum. Such alternations seemed to have almost the effect of continuous darkness and the plants were chlorotic. On the other hand, when the alternating light and dark periods were reduced to 15 seconds in length, growth increased, and extremely short alternations (5 seconds) had the general effect of continuous illumination.

Light thus exerts a double influence upon growth; it determines the rate at which the food is manufactured and also the rate at which it is used. An intensity which favors one may hinder the other, and much careful research remains to be done to determine the optimum combination of factors for each species of plant.

Light affects the germination of seeds in various ways according to the species. Some, like the grape, pine, and mistletoe, are favored in germination by light, while others, such as the jimson weed, germinate best in the dark. Still others such as chicory, parsnip, and parsley are unaffected by the light. It is thought that light aids those of the first group by hastening ripening processes which must be completed before germination can occur; and some seeds which ordinarily require light will germinate in the dark if iron salts, organic acids, alcohol, or other stimulants are supplied. As the temperature falls, the optimum amount of light increases, which indicates that the light acts partly through its heat or its energizing property.

The morphogenic effect of light is very pronounced. In the Bryophytes and Pteridophytes, light plays an important rôle in determining the early development of the plant. In *Equisetum* the first cross wall of the spore is formed at right angles to the incident light. In *Marchantia* gemmæ, the rhizoids always form on the shady side, and in fern prothallia the formation of the sexual organs on the ventral side is largely determined by light. Among the higher plants, the effect of light is plainly seen in the structure of the leaves. Plants growing in very bright light have several layers of palisade cells, while those in the shade have only one such layer. Similarly, erect leaves like the *Iris* have palisade layers on both sides. In the bluebell (*Campanula rotundifolia*), the leaves at the base of the plant in the shade are cordate with long petioles, while those along the stem in the light are linear with short petioles. If a plant with both kinds of leaves is grown in very dim light, the buds on the upper stem, which would ordinarily produce the linear leaves, develop into long-petioled, cordate leaves like those found normally only near the ground.

Moisture.—Water is absolutely necessary in the plant. It plays an important direct or indirect rôle in the various chemical reactions which take place within the plant, and it also provides turgor, which is a necessary physical condition. Directly, water takes part as one of the reacting substances in photosynthesis and in the various hydrolyses which take place within the organism. All the other reactions occur in a watery medium so it should not be necessary to emphasize further the part played by water (Chap. XX).

On the basis of their water requirements, plants are divided into three categories: hydrophytes, mesophytes, and xerophytes. The first named include such plants as water lilies (*Nymphæa*), *Elodea*, *Utricularia*, the algæ, etc., which require a great abundance of water. The mesophytes include the average plants, which get along on a medium amount of water. Xerophytes are built in such a manner that they can thrive, or at least live, on a very small water supply; they include the cacti, yuccas, and other desert plants (Chap. XX). Some of the so-called mesophytes are really *tropophytes*, i. e., they alternate between a hydrophytic condition in the spring and summer and a xerophytic one in the fall and winter when water is not available. There are also *physiological xerophytes* or *halophytes* found particularly in salt marshes, alkali regions, brackish streams, etc. These plants are living in a region where water is abundant, but, owing to its high salt content, they find it difficult to absorb. They hence often assume the fleshy, succulent appearance of many xerophytes.

It is possible for plants to get too much water especially at the fruiting season, when the fruit may take up so much water that it bursts and becomes unfit for sale. Water is also harmful to many plants like the date if it comes at the season of pollination, when the pollen grains, gorged with sugar, become so full of water because of their osmotic concentration that they burst and are valueless. The same damage may be caused to the pollen of many of the common fruit trees.

Water also exercises a strong morphogenic influence. Amphibious plants like *Proserpinaca*, which grows partly in water and partly exposed to the air, have finely dissected leaves in the water and flat, undivided ones in the air. The height at which the flat ones appear is determined entirely by the water level. The internodes in water are longer than those in the air, thus adapting the plant to the depth of the stream or pond. Houseleek (*Sempervivum*) in dry air is a rosette plant with succulent leaves. When given plenty of water and grown in a humid atmosphere, internodes appear and the leaves are more nearly normal, but these variations of form with humidity and transpiration have already been discussed (Chap. XX). In the case of apples, it has been noted that the lack of water makes the fruit more flat or compact and less elongated, so that even fruits may be changed by the water supply.

Owing to the close relation between light and transpiration, it may be difficult to separate an effect caused by light from one caused by humidity. The two factors work together to produce a common effect. Thus in *Convolvulus arvensis* Magocsy-Dietz (1920) found four distinct types of leaves depending upon the combination of the two factors of radiation and moisture: (a) auriculate or lanceolate leaves, in dry and moderately sunny places; (b) hastate, in shady and moderately damp places; (c) sagittate in bright sunlight; and (d) elliptical, in diffuse light and rather damp places.

Oxygen.—The effect of oxygen upon respiration has been previously described (Chap. XXIV). Here it only remains to add that Jaccard (1893) noted that any change in the oxygen pressure above or below normal stimulated growth, provided the change was not too great nor too prolonged, in which case growth was diminished.

The form of the plant, especially in the cryptogams, may also be controlled by regulating the oxygen content of the environment. In the common mold (*Mucor*), the hyphæ which form the ordinary mycelium break up into short cells resembling yeast if oxygen becomes deficient, thus forming the "mucor-yeasts."

Food.—The amount of food present is, of course, a factor in determining growth, but it has seemed advisable to consider this subject under the conditions which determine the manufacture of food,—light, water, etc.

Nutrient Salts and Ions.—The effects of the salts and their ions have already been discussed in the early chapters of this book (Chaps. IX and X); but, in this connection, attention should be called once more to the importance of hydrogen ions in the growth processes of plants (Chap. XI). This frequent resort to cross references should serve to emphasize the interrelation of all the processes met with in the life of plants. Although we may first discuss respiration and then growth, the plant does not respire and then grow; it does both simultaneously and interdependently.

Chemicals.—The essential elements are necessary for growth for reasons already given (Chaps. X and XI). There are also many compounds which hinder growth. Some of these like formalin, mercury bichloride, and copper sulphate have lent themselves to use as disinfectants, sprays, etc., because of their growth-prohibiting properties.

Many gases, especially illuminating gas and its chief constituents, ethylene and acetylene, have an injurious effect upon plants. These gases not only check growth but they also cause marked epinastic movements of the leaves and floral parts (Chap. XXIX) and an early dropping of the lower leaves. This is associated with the fact that the gases hinder the development of supporting tissues as well as of cutin and cork. Etiolated shoots are especially sensitive and may be used to test the presence of ethylene, which causes marked epinasty in tomatoes when present in only 1 part in 200,000 (Crocker, 1929). One can smell illuminating gas when 1 part is present in 400 of air, but many plants are damaged when ethylene is present in proportions of only one part to a million! In fact, Zimmerman reported that these gases were more dangerous to roses when present in these very low concentrations than in higher ones.

In smelter smoke, the destructive effects of which are sometimes observed for many miles from the source (50 miles according to unpublished work of Hedgecock), the most dangerous component is generally sulphur dioxide. In the many lawsuits that have resulted between growers and smelter owners, the latter have tried to prove that the damage was too far from the smelter to be caused by gases therefrom, but analyses of the air and leaves have generally shown the growers to be right. Plants should have as pure air as possible with a sufficient amount of carbon dioxide for maximum growth.

On the other hand, some gases (Chap. XXVI) have a stimulating effect upon growth and are used for the forcing of plants. Among these are the vapors of the common anæsthetics, chloroform and diethyl ether, as well as many others, including ethylene chlorhydrin, ethyl bromide, carbon bisulphide, etc.

Wounds.—Darwin (1880) noticed that plant roots when wounded slightly bend in such a way that the tip moves away from the wounded side. This has been interpreted as purposeful, but it is probably connected with the fact that wounding may result in greater respiration and greater stimulation of activity, which in turn produces greater growth on the wounded side, thus bending the tip away. If the wound is severe, growth is checked on the side of the wound and the bending takes place *towards* the wound. This fact alone is sufficient to overthrow the "adaptation" theory.

When stung by insects the curious formations known as galls occur. These differ in size and shape with the insect and the plant, and are so characteristic that *cecidology* has become a complex taxonomic science. Similar outgrowths may be produced by the attacks of parasitic fungi, among which are those causing the "witches'-brooms" found on ferns, hackberries, and ash.

The type of growth after wounding is of interest in phylogeny, and is thought by morphologists to throw light upon the ancestors of the plant. Wounding causes a return to an ancestral condition or the formation of atavistic structures which may be of extreme interest. Thus the flower heads of the hawk's-beard (*Crepis biennis*) when infected with the mite, *Eriophyes*, become transformed into umbel-like structures, which are interpreted as an ancestral type. Similarly the diœcious plant, *Melandryum album,* has perfect flowers when attacked by the smut, *Ustilago;* and the fir (*Abies*) produces resin canals when wounded. Such changes are all thought to be reversions to an ancestral form.

Mechanical Stimuli.—When pressure is applied to an organ, growth is retarded. When a bean root was placed in a gypsum cast for about a month, not only was enlargement checked but spiral and pitted vessels, which in ordinary roots are not found nearer than 3 cm. of the tip, were here found within 2 mm. A cross section from such a confined root taken 2 mm. from the tip has the same appearance as a section 30 mm. from the tip of a normal root. The stimulus caused by contact may also determine the location of organs. Thus the location of roots or tendrils on climbing plants is often determined by the places of contact between the climber and the support. Similarly the haustoria of the parasitic dodder (*Cuscuta*) occur where the parasite and host come in contact, and seem to be determined by the stimulus of contact or pressure.

Tension first retards the growth of the organ and then stimulates it. The retardation seldom lasts more than a day, after which growth is greater than normal. The increase of growth produced by a slight tension may be as much as 20% more than that of the control. The morphogenic effect is generally confined to the production of spiral thickenings on the vessels and a general increase in the firmness of the organs, which changes undoubtedly have survival value.

Gravity.—Gravity influences development chiefly in determining the *direction* of growth. For this reason a discussion of its influence is reserved for a later chapter.

Electricity.—The effect of electricity on plant growth has received much attention of late in an attempt to find some easily applicable method for the electrical stimulation of plants. Most of the work has been done either by placing seeds in an electrolytic bath or by placing the growing plant in an electric field produced by a silent discharge. The results obtained by various workers are often contradictory, but in general it can be said that the electrolytic treatment of seeds accelerates their germination and hastens the development of the seedlings. The treatment of the growing plants has generally resulted in earlier and better crops. Wheat, for example, when so treated produced an increased yield of 39%, and sugar beets were found to produce a 33% greater yield with 1.1% more sugar. Part of this additional growth may be due to the ionization of the air, as previously suggested (Chap. IV).

QUESTIONS

1. From the discussion of the morphogenic effects of temperature on barley, would you conclude that barley is a plant of a cold or warm climate?
2. If *Helianthus* seeds are heated to a high temperature, the seedlings often develop abnormally. Is this a "morphogenic" effect?
3. Give two reasons why red light is better than blue light for the growth of plants.
4. What would be the effect of the half-shade system of propagation upon vitamin content?

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CHAPTER XXVIII

IRRITABILITY; POLARITY AND CORRELATION

A sensitive plant has no business growing in a public highway.
—S. COULTER.

Heredity and Environment.—The growth and development of a plant are determined by two separate sets of factors. First, there are those of *heredity*, which the plant inherits with the sperm and egg, if it is the product of sexual reproduction, or which it obtains from the one parent if produced asexually. Secondly, are the factors which are not hereditary but which make up what is called the *environment* of the plant. Although this term is generally used to include only those factors which are external to the plant, if we include everything which is not inherited as environmental, then we must also speak of the *internal* environmental factors as well.

Stimuli and Responses.—Organisms are very sensitive to changes in the environment. Changes in temperature, moisture, pressure, etc., soon make themselves felt, since the protoplasm is sensitive to all these environmental factors and to changes in them. Environmental factors which cause the plant to alter its growth and development are called *stimuli*, among the more common of which are light, water, heat, etc., the effects of which have already been discussed. The change which the plant makes in order to adjust itself to the different environment is called the *response*. The response is not always visible since it may be an internal change which cannot be detected from the exterior. The sight of food may cause an animal to *move* toward the food or it may merely cause the animal to *desire* the food. Both are responses but only the former is visible. In the case of the desire there have been rearrangements within the nervous system, but it may happen that no external sign indicates these changes. The response may also be purely mechanical as when a gust of wind blows the branches of a plant, but these mechanical responses are not the ones usually meant when stimuli and responses are discussed.

This power which organisms have to adjust themselves to

DR. J. B. OVERTON, Professor of Plant Physiology, University of Wisconsin, Madison. *Ascent of sap; Root pressure.*

SIR J. C. BOSE, Director of the Bose Botanical Institute, Calcutta, India. *Mr. Bose is most favorably known for the delicate instruments which he has devised and applied to the study of plant physiology, especially to the phenomena of irritability and conduction of stimuli.*

their environment by responding to changes in moisture, heat, light, etc., is called *irritability*. A living plant or animal is consequently irritable. A tree growing in a wood is subject to a flood of stimuli: the light which falls upon it is varying in direction and intensity through the day and from season to season; the moisture content of the soil and air varies from a comparatively small amount on a dry day during a long drought to saturation during a rainy spell; and in the summer the temperature is high, while during the winter the water between the cells may be freezing. If all these many factors are combined in all the possible ways, one may get some conception of the varying conditions under which a plant carries on its work. To adjust itself to all these changes, is the chief problem of living protoplasm. Under the normal conditions, when these factors are well combined, the organism is thriving and in good health. If *life* means the adjustment of the organism to the environment, then *sickness* and poor health are accompanied by a partial loss of irritability as the organism loses the power to make the proper adjustments under unfavorable conditions, and *death* is the permanent loss of irritability. Anæsthesia, in which certain phases of irritability are temporarily but not permanently lost, should not be confused with the state of "suspended animation" as seen in seeds and trees in winter. In these latter cases, the plant is inactive, but this inactivity is in itself a form of response; as soon as water, heat, etc., are present, the plant becomes as active as ever. In anæsthesia and death, the power to respond is gone; in suspended animation the power to respond is as great as ever.

Fatigue, Tetanus, and Rigor.—If an organ is stimulated and the stimulus is withdrawn, the organ will respond and then return shortly to the normal condition, when it is ready to respond again. If several stimuli are given in succession before time for complete recovery has elapsed, the response diminishes. The organ is becoming *fatigued* and lacks the power to respond as at the beginning. This condition of fatigue may continue, if the stimuli are repeated, until the organ lacks the power to respond at all. Unlike the original unstimulated position, it assumes a rigid fixed one from which no stimulus can arouse it. This condition is that of *tetanus*, and when this has set in a long rest is required for complete recovery before the plant or organ becomes again irritable or sensitive.

If a plant is exposed to too high a temperature, it does not respond normally to stimuli and is said to be in a condition of *heat rigor*. In a similar manner a state of cold rigor, of drought rigor, of darkness rigor, etc., may be induced. If the state of rigor is not too prolonged, the original irritability will be resumed when the plant is restored to normal conditions.

Characteristics of Irritability.—Irritability is characterized first of all by a delayed response. The response to the change in the environment is not immediate; it takes some time for it to be made evident. Secondly, there is more energy used up or expended in the response than was expended in the original stimulus. When a boy is pricked with a pin, the amount of energy spent in pushing in the pin is very small compared with that with which the response is shown when *stored* energy is released. Similarly in plants, the amount of required energy expended by light on a stem is less than that which the plant spends in bending toward the light. Thirdly, the response of plants and animals to stimuli is generally (but not always) of such a nature that it serves a purpose. That is, in the course of evolution organisms which responded as the present ones do, had a better chance of living in the struggle for existence. The responses have thus had survival value.

The delay of the response is the result of its three phases,—the reception of the stimulus, the transmission of the stimulus, and the final reaction or response proper. The receptive region is not the same as the responding region. Animals receive stimuli through the afferent nerve endings and respond through the muscles and other parts of their effector systems. Plants may also respond in a place very different from where the stimulus is received. In the case of animals the receptive regions are generally very specialized; the eyes receive light stimuli, the ears sound stimuli, etc. In plants such localized receptive regions for special stimuli are rare; the entire surface is more or less sensitive to all stimuli. In other words, the reception is *diffuse* rather than localized.

The question of the transmission of stimuli has always much interested physiologists. In animals, the nervous system serves to transmit stimuli, but in plants no analagous nervous system has been recognized. Bose (1925) of India has asserted that the conducting strands of protoplasm in the phloem are specialized for the conduction of stimuli and have the same function as animal nerves, but his ideas on these questions have not yet had a large following in the Western World. Response in plants is generally much slower than in animals, indicating that the channels of transmission are not so efficient. Each cell in the plant transmission chain is much shorter than the nerve cells with their neurons, with the result that the stimulus cannot travel far in one cell before it must cross the boundary of the adjacent one. The stimulus travels much like the impulse one sets up when tumbling down a row of blocks. As each block falls it impinges upon the next one, which then transmits the impulse given it to the next, and so on until the end block is overturned. There is thus a series of stimuli and responses as the original stimulus travels down the transmission line.

The Response.—By the "response" is generally meant the first visible end response. The change within the cells during the transmission is a response to the original stimulus, but this reaction (which is not the end one) is not easily seen and is not the response meant. When light acts upon a stem many changes may take place within the cells before the stem actually starts to bend towards the light, but only this final bending is included in the visible response.

The same stimulus may bring about different reactions or responses, depending upon the machinery. By pressing a button one may ring a bell, turn on a light, start a motor, etc. The stimulus of pressing the button is the same in all cases, but the response is different because the apparatus is very different. Similarly in plants, the stimulus of electricity may cause a stamen to shorten, the leaf of a sensitive plant to drop, or a tendril to curl. Gravity may cause stems to go up and roots to go down. In all these examples the stimulus may be the same, but it operates through a different mechanism and results in different responses.

Reaction Time.—A stimulus presented for a very short time may bring about a change in the cells in the immediate neighborhood without ever causing any ultimate visible reaction or response. To bring about the response, the stimulus must be presented for a minimum time, which is called the *presentation* time. After the presentation of the stimulus, it requires some time for the stimulus to be transmitted. The *transmission* time is relatively short (a few seconds or minutes), but before the response can be seen the cells in the reacting region must have time to react. The response is commonly seen in some form of growth and this requires time. The reaction time is thus the sum total of the presentation time, the transmission time, and the growth time. Many organs are more sensitive at certain times and under certain conditions than at others. When *Osmunda cinnamomea* fronds are maturing, they will respond to gravity after a presentation time of only 20–40 seconds.

If the stimulus is increased in intensity, the time required for presentation is correspondingly shortened so that *the presentation time varies inversely with the intensity of the stimulus.* A strong light acting for a short time is just as efficient in evoking a response as a weak light acting for a long time. The law of summation then follows: A stimulus too slight to produce the end reaction, if repeated at proper intervals, will ultimately suffice to cause the end reaction.

Weber's Law.—If a change in the environment is great enough to produce a response, it has been found that the necessary change bears a certain relation to the original stimulus. Let us suppose that the plant is receiving 100 units of light on all sides and it is desired to increase the light from one side so as to cause a bending. It may require a difference of ten units, i. e., one side may have to receive ten units more than the others before any bending is observed. If this is the case, and it is desired to bend a plant which is receiving 200 units, then one side would have to receive *twenty* more than the others. This is expressed by Weber's Law: "The smallest change in the magnitude of a stimulus which will call forth a response always bears the same proportion to the whole stimulus." In the example given the proportion was 10%.

Two Stimuli from Different Directions.—When two stimuli act upon a plant tending to cause a response in different directions, the organ affected will assume a position intermediate, according to the laws of forces. If the force exerted by each stimulus is represented by one side of a parallelogram, the force actually acting upon the organ will be represented by the diagonal of the parallelogram, as described in any elementary textbook on physics. In the same way, if two forces are acting in directly opposite directions, the ultimate reaction will correspond to the difference of the forces acting; if operating in the same direction, the response corresponds to their sum.

Kinds of Stimuli.—There are many different kinds of stimuli acting upon the plant. Among these are light, water, wind, gravity, heat, the chemicals of the soil, electricity, pressure, etc. Barnes (1910) attempted to classify these various stimuli as mechanical, chemical, and ethereal, but since the nature of many of these stimuli is as yet unknown, any kind of classification is open to question. His classification may serve as well as any.

Geotropism.—As an illustration of the reactions of the plant to stimuli, the response to gravity will be discussed more fully. It has long been noticed that plants grow in such a way that stems go away from the earth, roots grow towards the earth, and secondary branches and leaves grow out at various angles. Roots, which go towards the pull of gravity, are said to be *positively* or *progeotropic*, stems are *negatively* or *apogeotropic*, while leaves and branches which grow at right angles to the force of gravity are *plagiotropic*. Certain roots and branches set themselves at a definite angle (other than 90°) to the force of gravity or remain at a certain depth in the ground. Such organs are *diageotropic*.

That gravity is actually the cause of the position assumed by these organs can be shown by (1) either neutralizing the effect of gravity or by (2) replacing it with some other force. In the first type of experiment, plants are placed upon a *slowly* revolving wheel or disk called a *clinostat*. If a plant is revolved horizontally on such a wheel rotating parallel to the long axis of the plant, the roots and stems continue to grow in the direction in which they were growing at the beginning of the experiment. The stems do not turn up nor the roots down. Gravity here has a chance to act on all sides of the stem equally, and the result is an equal growth of all sides. If on the other hand a plant is laid horizontally without rotating it, the stem will soon turn up and the roots down in response to the influence of gravity.

In Knight's experiment, the young plants are placed upon a *rapidly* rotating disk so that the centrifugal force produced is much greater than the pull of gravity. In this case the stems turn toward the center of the wheel *away from* the pull of centrifugal force, while the roots grow *toward* the circumference in the direction of the pull. Here gravity is negligible as compared with the much larger force exerted by the rotation. In both these experiments, evidence is produced which leads to the unavoidable conclusion that the direction of the growth of the plant organs is largely determined by gravity. That gravity is acting as a *stimulus* and not as it might act upon dead, inert matter is shown by the fact that the stems go *up.* If it were merely a question of weight, the stems would go *down* towards the pull.

The Perceptive and Responsive Regions.—The region sensitive to the pull of gravity in the root, is located in the last two millimeters including the rootcap, the inner portions of which are believed to contain the cells most concerned in the reception of the stimulus. That this is so can be shown by cutting off the end two millimeters, when the root no longer responds to gravity. It is also possible to place the tip in a small glass slipper as done by Czapek and to turn the tip so that it alone is at right angles to gravity. In this case the root bends in such a way as to bring the tip parallel to the gravitational stimulus. If, on the other hand, the tip is kept parallel to the force of gravity and the rest of the root is bent at right angles to the pull, no effect is observed (Fig. 23). The effective force acting upon a root placed at an angle, A, with the vertical is equal to the sine of the angle. Since (see above) the active force multiplied by the perception time is a constant, the perception time varies inversely with the sine of angle A (the larger the angle the greater is the pull of gravity) (Fig. 24).

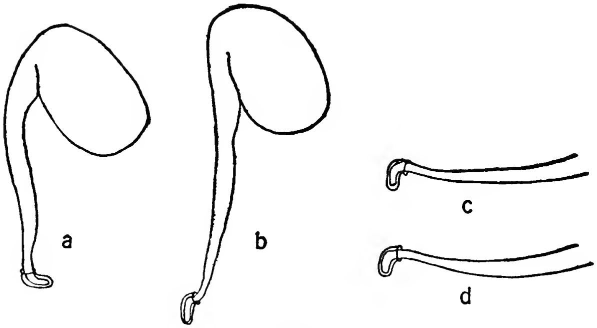

FIG. 23.—Roots of horse bean (*Vicia*) in glass slippers. If the terminal 2 mm. are bent at right angles to the perpendicular as in a, the root will turn as in b, so that the tip is again vertical. If the tip is vertical it makes no difference if the remainder of the root is not, as shown in c and d where no change takes place in position. (After Czapek.)

The region which responds is located back some distance from the tip in the section where the greatest growth occurs (3–4 mm. from the tip). The transmission region is consequently very short, since the growing region is directly behind the receptive region.

The Statolith Theory.—Precisely how the plant is able to perceive the stimulus of gravity, has been the subject of much research. When organs bend under the stimulus of gravity or light, there is found on the convex side (1) increased respiration, (2) increased acidity, (3) increased hydrolysis of polysaccharides, and (4) increased turgor. These various changes are to be considered, however, as effects or accompaniments of the bending rather than as causes.

Growth hormones have been offered by Loeb and others as an explanation of the common tropisms; and while the hormones may be associated with the *growth*, the more immediate cause of the *bending* is now attributed to other factors.

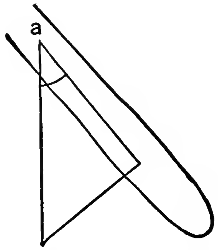

FIG. 24.—The effective stimulus on a root varies with the sine of angle a, i. e. the angle which the root is bent from the vertical.

Němec (1902) and Haberlandt (1903) proposed that the response is called forth by movable starch grains in the receptive regions. When the root is growing vertically (normally) these starch grains or *statoliths* are resting on the lower ends of the sensitive cells (*statocysts*) which contain them. If the root is tilted out of this position, the statoliths fall on a sensitive portion of the protoplasm not accustomed to them, and the mechanism which brings about the end response is put into action, resulting in the return of the statoliths to their normal position at the lower end of the cell. While this theory began as a purely speculative hypothesis, much work has been done to confirm it. The sensitive regions of root and stem contain cells rich in starch which meet all the requirements of the theory. In *Asplenium bulbiferum* the statocyst tissue arises during development and then disappears parallel with the geotropic sensitivity; similarly, the starch grains in the endodermis of *Tagetes* disappear in the dark and also the geotropic sensitivity (Zollikoffer, 1918). Prankard, who has developed the original theory more than anyone else, has found that objects other than starch grains may serve as statoliths. In wheat culms crystals of calcium oxalate serve this purpose, and when thrown out of their original position they fall in the cell at the rate of ten microns per minute, which may explain the extreme sensitiveness to gravity of the wheat and related cereals. Chloroplasts (*Ophioglossum* and *Lunaria*) and movable nuclei have also been suggested as statoliths. In support of this view is the work of Henckel (1926), who found that the vertical position of the filaments of the alga, *Enteromorpha intestinalis*, is associated with a definite geotropic position of the chloroplasts. This type of evidence has been multiplied to the point where to speak of the statolith "hypothesis" or "theory" is almost misleading.

Czapek (1906) has rather opposed the theory in favor of a chemical explanation, since he finds that poisonous gases cause a loss of sensitiveness to geotropic stimuli. This, however, seems no argument against the statolith theory because we know that between the reception and the response come many chemical processes which might be affected by poisonous gases. Poisoning by gas sheds no light on the statolith mechanism whatever. The statolith theory, in addition to affording a beautiful explanation of a hitherto mysterious phenomenon, is interesting in that it affords in plants a mechanism analagous to the otocysts of the Crustaceæ and to that of the semicircular canals of the inner mammalian ear, by which these animals maintain their equilibrium.

Twiners.—Among the most interesting stems from the point of view of their relation to gravity are the twiners. When the young plant grows up from the soil, the first internodes are generally erect. Each new internode then elongates rapidly and the leaves of the youngest internodes are retarded in their development, leaving bare the tip, which no longer grows erect but declines to one side and begins a very marked regular movement. The tip, which is now nearly horizontal, swings steadily around in the direction of every point on the compass. If this movement brings it in contact with a support, the stem then proceeds to twine about it, each new twist being at a higher level than the preceding. In some plants the direction is to the left and in others to the right.[1] There is no inherent reason why a

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[1] In naming twiners there is much confusion. Some have considered those as *dextrorse* which turn to the right across the support. Others have given this name to those which turn *clockwise* as seen from above looking down on the plant. Obviously the one is just the reverse of the other. The same is true for *sinistrorse* or left-handed twiners.

plant should turn in one direction more than in another, and, while the members of the same species usually twine in the same direction, closely allied species may twine in the reverse fashion. There are some species which twine in either direction, and, in a few cases, the individual plant may change its direction during the course of its development.

If the plant is rotated on a clinostat, the twining ceases, showing that the growth of one side more than the other, which produces the twining, is in some way related to the stimulus afforded by gravity, although the progress of this growing region about the stem, now on this side and now on the other, is regulated internally. There are details in the mechanism of twining which still remain to be solved, although negative and lateral geotropism are known to play a very important rôle.

The Sensitive Plant.—*Mimosa pudica*, called the sensitive plant, is incorrectly named because it is not more sensitive than other plants. Every plant is a sensitive plant; if it were not it would be dead. The difference lies in the fact that *Mimosa* seems to have an exceptionally rapid transmission mechanism and an end response which may be much more clearly seen than in most plants. In this member of the Leguminosæ the leaf is twice compound. The petioles and stems contain an abnormally large cortex as compared with the wood and pith, and at the base of each petiole and petiolule, outside the vascular tissue, is an extra layer of parenchymatous tissue which makes a swollen place called the *pulvinus* (Fig. 25). These pulvinus cells are more than ordinarily large, are rich in water, and are interspersed with abundant intercellular spaces.

If the leaflets are stimulated by pressure, heat, anæsthetics, etc., they quickly droop. This stimulus from the leaflets is rapidly transmitted at the rate of 1–3 cm. per second to the large pulvinus at the base of the petiole, when the entire leaf then droops. The stimulus may even travel both up and down the stem to the adjacent leaves. After a time the normal position is resumed.

The drooping is associated with changes in the tissue of the pulvinus, the cells of the lower half of which are thinner walled and have more intercellular spaces than those in the upper half. If the lower half is cut through as far as the vascular tissue, the leaf falls never to rise again; while if the upper half is cut, the leaf falls but rises higher than ever, which shows that the lower half of the pulvinus is most concerned in the movements. The rise to a higher position after cutting the top part, is due to the fact that it is not restrained by the tension in the thicker-walled cells of the upper half, which are no longer functional.

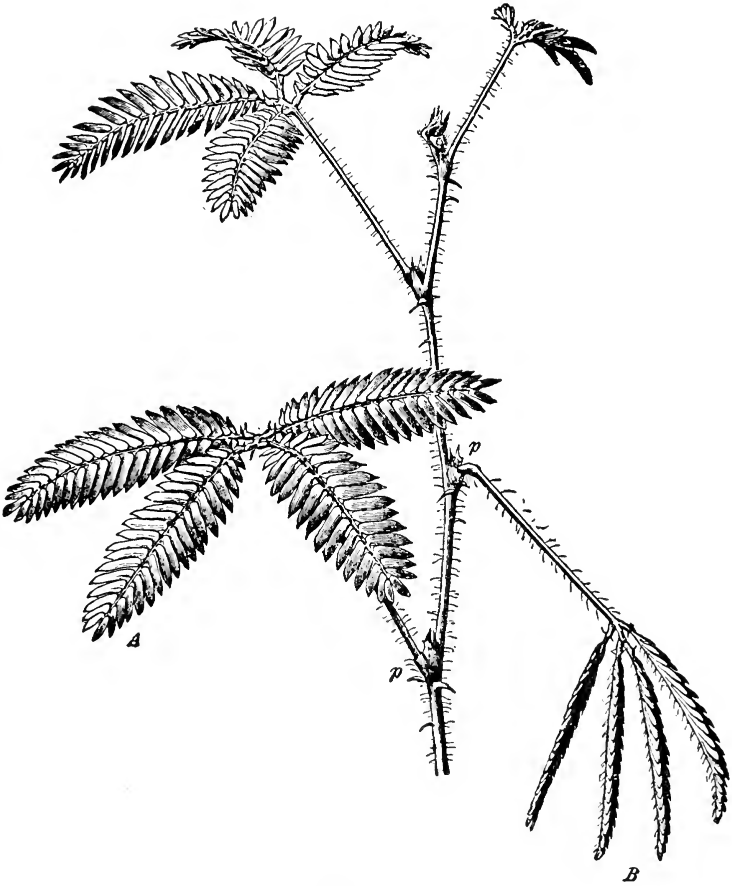

FIG. 25.—The sensitive plant (*Mimosa*). *A* is the normal position of the leaves and *B* is the position when touched, burned, etc.; *p* is the pulvinus. (After Pfeffer.)

The drooping of the leaf is due to the loss of water from the cells of the pulvinus into the intercellular spaces. This causes a loss in turgor with the resultant fall. Later the water is resorbed from the intercellular spaces and the leaf assumes its normal position. How these changes in the permeability of the cells are brought about still remains to be solved. That the drooping is not a mere pull of gravity is shown by the fact that if the plant is inverted and stimulated the leaves fold *up* towards the roots. When the turgor is released on the basal half of the pulvinus, the tissue tension on the apical half consequently *forces* the drooping of the leaves.

The past few years the interest in this plant has centered largely on (1) the region of the stem and petioles in which the stimulus is transmitted, (2) whether living cells are necessary for the transmission, and (3) whether the stimulus is hormonic or nervelike in nature. Among the chief workers in the field have been Haberlandt, Bose, Ricca, Ball, and Snow. Haberlandt thought the stimulus was carried by water waves in the elongated cells of the phloem. Ricca (1916) found that the stimulus would travel through the stem even when the stem was girdled for six inches or if killed by heat, and he concluded that the xylem was the chief conducting region. When cut in two and separated by water in a glass tube, the stem below exuded a greenish substance, which passed through the tube and caused a reaction in the part above. Snow (1925) concluded that the stimulus traveled in the xylem in the stem but transferred to the phloem at the pulvini of the leaves, continuing from there in the phloem. Ball (1927) found two types of conduction—one at a rather slow rate (20 cm. per minute) in the xylem, and a more rapid or "explosive" type (200 cm. per minute) through the pith. In this latter case, the conduction of the stimuli was associated with the collapse of the turgid pith cells, which as they contracted seemed to eject a substance that caused the contraction of neighboring cells. These facts and theories all point to a hormone traveling in the transpiration stream, but objections to this conception are the facts that the impulse travels in *both directions* (up and down) and *faster* than the transpiration stream. Bose, from a series of elaborate experiments, came to the conclusion that the transmission is in the phloem and by some mechanism similar to that in the nerves of animals. The famous Austrian physiologist, Molisch, went out to India as a sort of representative from the West to see just how much Bose was enlarging his results with the very sensitive auxographs he has constructed, but, much to the surprise of the Occident, Molisch became a convert and has supported the main contentions of Bose. It would seem possible, however, that this "nervous" reaction measured by Bose and Molisch is the rapid or "explosive" conduction of Ball. Probably the stimulus travels mostly in the pith and xylem in the stem, as stated by Snow, and changes over to the phloem in the petioles, where the hormones in question are aided by the same diffusion processes of Mason and Maskell (Chap. XXIII) so useful in the translocation of foods. Thus we are able to harmonize to some degree the contributions of these various workers.

Polarity and Correlation.—In Chapter XVII, attention was called to the fact that in stems one end is associated with the formation of leaves and branches while the other end forms only roots. This tendency to form organs of a distinct kind at the various ends or parts, is described by saying that the organism shows *polarity;* this is not an explanation but merely a restatement of the phenomenon. After the polarity has once been established it is generally difficult to change, although in some forms like *Bryopsis* (one of the Algæ) the polarity may be reversed after it has once been determined. In Chapter XVII these phenomena were explained as due to diffusing hormones, but other explanations, in some cases more concrete, have been offered. Thus Davies and Hicks have shown that in the case of willows, which develop shoots from the upper end and roots from the lower, this polarity is connected with the ratio of the carbohydrates to the nitrogen compounds (the C:N ratio). The shoots tend to form where there is the largest amount of nitrogen, i. e., where the C/N ratio is lowest, and roots from places where there is less nitrogen and more carbon compounds.

By *correlation* is meant the reciprocal influence of organs. In addition to the examples cited in Chapter XVII, a few others may here be mentioned. Among the frequently observed *quantitative* correlations is the relation between blossoms and vegetative parts in the sweet pea (*Lathyrus*). As long as the flowers are removed, new buds and blossoms are produced; otherwise blossoming stops and fruiting begins. Fern gametophytes live ordinarily only until the sporophyte is well established, but if fertilization is prevented, the death of the prothallus may be delayed for months, with the production of new archegonia. The production of blossoms and fruit on fruit trees may be increased in this same fashion by judicious pruning.

Among the *qualitative* correlations is that between the main and lateral axes of Gymnosperms (Chap. XVII). Another equally interesting example is seen in the potato which produces normally two kinds of stems: the aërial, leafy ones and the subterranean ones which bear the tubers. If the aërial stems are cut away, one of the subterranean stems will turn up into the air and develop leaves and branches. If all the underground buds are removed so that no stems are present below ground, tubers will form on the lower part of the stem above ground. These tubers are similar to underground ones, but are bright red in color and bear leaves at the eyes. If the top of the stem is darkened, the tubers will form at the apex, showing that the translocation of food is here up instead of down, in which case the polarity has also been reversed to a certain extent. Such experiments serve to show the close connection between correlation and polarity, but these problems are all very complex and are tied up with the whole question of regeneration and growth, upon which much work remains to be done.

The relation between polarity and correlations is well illustrated by the recent work of Lund, who has found that the cells of onion roots, *Bryophyllum* leaves, and Douglas fir shoots generate electric currents continuously. The electric polarity of each cell is oriented in such a fashion that the cells act like a series of batteries. Their respective individual E.M.F.'s are added and thus may build up a considerable difference in potential. In some cases, however, the currents developed may oppose and neutralize each other. In the Douglas fir, the apex of the stem acts as a positive pole in respect to the base of the stem and the apex of the lateral branches. This electrical dominance of the main stem seems to be correlated with the physiological dominance of the main axis; and Lund believes that electric currents flow constantly along certain circuit channels of the tree and thus unify and correlate the living regions into a definite electrical system.

QUESTIONS AND PROBLEMS

1. Of what "survival value" is the positive geotropism of the root system?
2. If a weight of one pound is held in the left hand and it requires a weight of 1 lb. 2 oz. in the right before an increase can be detected, what must the weight in the right hand be if the weight in the left is 3 lb. 8 oz.?
3. Gravity is attracting a root with the force X and moist soil at one side

is attracting it with the force Y. Show by a diagram the direction of the growth of the root and the magnitude of the resultant force acting upon it.

4. How does the explanation of Child for the correlation phenomena differ from that of Loeb?
5. How can it be proved that geotropic response is not due to weight?
6. Name a twiner of each class mentioned in the text. Give an example of a species which twines in either direction and one which may reverse its direction.
7. Is oxygen necessary for geotropic responses?

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CHAPTER XXIX

MOVEMENT

> All things work and move.
> —J. S. Dwight.

All plants possess to a certain degree the power of motion, and the following classification of the various movements exhibited by plants is as complete as our present knowledge permits. Each type of movement will be discussed briefly in turn.

- I. Active or spontaneous movements.
    - a. Internal
        - 1. circulation
        - 2. rotation
        - 3. streaming
        - 4. orientation
    - b. External
        - 1. change of position
            - A. through growth
            - B. through independent locomotion (taxies)
                - a. amœboid movements
                - b. ciliary movements
                - c. excretory movements
        - 2. no change of position—curvature
            - A. due to imbibition
            - B. due to turgor changes
                - a. autonomic
                    - 1. nutations
                    - 2. nastic movements
                - b. paratonic
                    - 1. nastic movements
                    - 2. shock movements
            - C. due to permanent growth
                - a. autonomic
                    - 1. nutations
                    - 2. nastic movements
                - b. paratonic—tropisms
- II. Passive movements—caused by wind, blows, etc.

Internal Movements.—The internal movements of the cells are visible only with the microscope, but are more or less characteristic of all living cells. Cytoplasmic motion, which is an attribute of all active cells, is manifested either by isolated streaming movements, which may be reversed, now going in this direction and now in that, or by a single stream, whose direction is constant. These two forms of movements are known as *circulation* and *rotation* respectively. In circulation the motion is observed not only in the layer of protoplasm lining the cell wall but in the various strands of cytoplasm which cross the vacuole, while in rotation the motion occurs only in the cytoplasmic layer lining the wall. Circulation is very common in land plants and may be easily observed in the staminal hairs of the spiderwort (*Tradescantia*), while rotation is more often seen in water plants such as *Nitella* and *Elodea*. The movements may be normal or they may be due to excitation produced by abnormal conditions. Rusk (1920) and Seifriz (1922) have reported that zinc, lithium, and other salts cause very rapid circulation and rotation of the protoplasm, which fact may be used to advantage for demonstration work.

In very large cells or cœnocytes, as found in the slime molds (*Myxomycetes*), *Rhizopus*, and *Vaucheria*, the protoplasm may move down through the center of the cell and back along the edges much as a current moves in a closed pipe. Such movement, closely related to rotation, is called *streaming* and may help to distribute the food and other materials within the cell.

Certain portions of the cell (nucleus or plastids) may orient themselves inside of the cell in response to external stimuli. Thus the chloroplasts move about within the cell in order to be in the most favorable place for manufacturing food. In very bright light they place themselves where they will not be injured by it, while in weaker light they come nearer to the surface of the leaf. This is seen to especial advantage in the cells of moss leaves and fern prothallia. In moderate light the chloroplasts gather along the walls at right angles to the incident rays, but in intense light

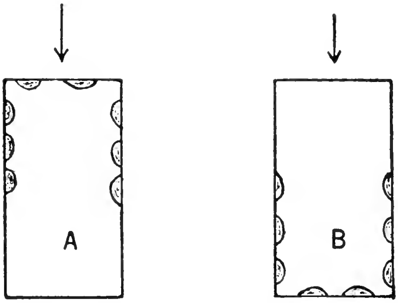

FIG. 26.—Orientation of chloroplasts in the light. A shows the position of the plastids in weak light. As the light becomes stronger the plastids of the palisade cells move toward the end away from the light as shown in B.

they are found along the walls which are parallel to the rays of light, thus protecting themselves as much as possible from its harmful action (Fig. 26).

External Movements; Change of Position.—The externally visible movements of plants are those commonly included in a discussion of motion. They result, either in an actual change of position, if the organism is not fixed to the earth, or to curvatures, in case the plant is attached in such a way that the entire body is not free to move. This movement of the entire organism may be due to the peculiar methods of *growth* of the plant or to independent *locomotion* similar to that of animals. Those organisms which move through growth generally possess underground stems which run parallel to the earth, sending down roots into the soil and sending up aërial shoots to procure access to air and light. The stems frequently die off behind as growth continues, with the result that the plant slowly marches across the terrain as the forward end grows and the posterior end disintegrates. A fern may move in this fashion five cm. a year, which makes a meter in twenty years. Although this rate is rather slow, time is not of paramount importance to a fern. *Iris*, *Nymphæa*, and others may move in this same manner.

Independent Locomotion.—The independent movements in plants are only to be found among the simpler forms. As stated in Chapter VII, animals and plants have evolved along two different lines,—the animals towards motility and the plants towards stability,—these differences being associated with the manner of procuring food.

Three types of movements are to be found,—amœboid movements, ciliary movements, and excretory movements. The first derive their name from the *Amœba,* an animal which exhibits better than plants this motion characteristic of the simplest organisms known. The chief plants to show this type of movement are the plasmodia of the Myxomycetes which, because of their many animal characters, are sometimes included in the animal kingdom. These organisms are *naked* masses of protoplasm which live in wet places on decaying wood, wet leaves, etc. When locomotion occurs, the lobes of the protoplast are advanced along one side forming a protrusion into which the rest of the protoplasm slowly streams. In this way the entire mass advances in the direction of these protruding lobes or pseudopods.

Free-swimming movements by means of cilia or flagella are seen in the swarm spores of many of the Thallophytes, in bacteria, and in the sperms of plants as advanced as the Gymnosperms. The cilia are protoplasmic structures which extend from the cell and produce movement by lashing the surrounding water. The swarm spores of *Fuligo* may move a millimeter in a second. This is sixty times their length and, when it is recalled that a good runner goes only 100 yards in ten seconds or five times his length per second, the speed of *Fuligo* swarm spores is not to be lightly regarded. The cholera *Vibrio* moves a millimeter in twenty-two seconds, which is also very rapid considering the size of the organism.

The most puzzling of the free-swimming movements are the excretory movements as seen in Desmids and Diatoms. These forms possess no visible cilia and yet they are able to move at a very high rate. Although their motion is not completely understood, it seems to be due to the excretion of some sort of mucilaginous material against the water through which they are gliding. Others (Müller) have thought that protoplasmic protrusions were thrust against the surrounding medium or substrate to produce the mysterious, majestic motion which these forms exhibit. The forward movement of *Oscillatoria* seems to be due to some similar push exerted by the secretion of something against the substrate, connected with high osmotic pressure (Krenner, 1925) in the motile filaments.

Taxies.—Only a comparatively few plants show the independent locomotions thus enumerated, but this does not mean that such motions are unimportant to the plants which possess them. While the *intensity* or rate of movement is affected by the general conditions which determine optimum growth and activity, the *direction* is also controlled and regulated by external agents. Such directed movements are called *tactic* motions or taxies. Among the external factors which influence these movements, several of the more important and the responses evoked, will be briefly described.

Phototaxy is the response of free-swimming organisms to light. If *Volvox* or the zoöspores of some species of *Chlamydomonas*, *Euglena*, or other Algæ are illuminated from one side with a light of moderate intensity, they swim toward the light and get as near to it as possible, i. e., they are positively phototactic. If the light becomes too strong, they become negatively phototactic and swim away to the opposite side of the container. Blue light is more effective than red in producing these reactions.

By *topotaxy* is meant the orientation and movement as affected by differences in the concentration of a substance. This may have no relation to the nature of the substance, i. e., it is not a question of quality but only of quantity. It is thus hard to distinguish from *osmotaxy*, where the movement is due to changes in osmotic pressure, which is also a quantity relationship.

In *chemotaxy* we are dealing with a *qualitative* difference which the organism is able to detect. Some substances produce positive chemotaxy, others negative, and to others the organism is indifferent. The responses may occur to substances which the plant would never meet in nature and in many cases they have no apparent survival value. On the other hand, the positive chemotaxy exhibited may be extremely helpful in some cases. The sperms of ferns are attracted by minute quantities of malic acid, which the archegonia excrete, and thus fertilization is insured. The amount of a substance required to produce the response is very small. A 0.001% solution of malic acid attracts the fern spermatozoa, and, while it is estimated that the amount which stimulates any one sperm is about one-millionth of the weight of the sperm, this is still ten times as much as the relative amount of morphine which is required as the minimum effective dose for man. *Aërotaxy* and *hydrotaxy* are special forms of chemotaxy.

Rheotaxy is the response made to a current of water. Many free-swimming organisms swim against the current, i. e., they are negatively rheotactic.

By *thigmotaxy* is meant the tendency to remain attached to the object around which the organism is swimming. When sperms of *Fucus* come in contact with an egg they swim around and around in close proximity to the egg until one has penetrated and fertilized it. This may be due to some substance secreted by the egg, in which case it would be a form of chemotaxy.

Some forms prefer to stay at a certain level in the water. If too near the surface they swim down, and if too near the bottom they rise until they reach their normal level. Such movements are *geotactic.*

The response to heat or *thermotaxy* is also very easily observed. If one side of a vessel of water which is just above the freezing point is warmed, the free-swimming forms will move towards the warm side. As the heat gets greater and greater, a time comes when the movement becomes negatively thermotactic and the organisms swim towards the cooler side of the vessel.

Loeb has explained these phenomena on the basis of symmetry. In thermotaxy, for example, as the vessel is warming up, an organism which is bilaterally symmetrical is warmed more on one side than the other. Reactions on both sides tend to go on at an equal rate, with the result that the organism turns itself in such a manner that both sides may warm up at the same rate. If it is swimming and headed toward the source of heat, its movement is destined to guide it directly toward the heat, since only when going either directly towards or directly away will both sides be receiving the same set of stimuli. This may explain many of the phenomena observed, but does not adequately explain the reversal from a positive to a negative response. This explanation has done much, however, to explain instincts on a purely mechanistic basis and to show why some instincts, e. g., the flight of the moth to the flame, may be harmful rather than purposeful.

Curvature Movements; Imbibition. The curvature movements of plants are due either to imbibition, to changes in turgor, or to actual growth in the usual sense. If imbibition and increase in turgor pressure are also growth (Chap. XXVI), then it can be said that all curvature movements are due to some phase of growth. If an organ bends, one side must get longer than the other whether the increase is due to imbibition, osmosis, or increase in the number of cells.

The movements which are due to imbibition are hygroscopic movements which are purely physical in character but which, in some cases, must be of much importance to the plant. In Chapter XIX it was shown that in imbibition water forces its way between the particles of material which make up the cell walls and drives them apart, increasing the size of the imbibing body. Similarly when water is lost, the particles come closer together and the body shrinks. If the one side loses or gains water more rapidly than the other, curvature will result.

The fruit of *Erodium* has a long slender beak, which extends straight out from the seed when moistened. When it loses water, one side of this beak dries out much faster than the other. Torsion results and the beak forms a spiral coil which helps to bury the seed in the earth. The bursting of seed pods when they dry out is due to similar torsions set up by unequal water losses. The elaters of the horsetails (*Equisetum*) and the teeth of the peristome of the mosses function in the same way, thus helping to scatter the spores. Very rapid hygroscopic movements of a similar nature occur in the bracts of *Ammobium*, *Rhodanthe*, and other Compositæ (Uphof, 1924).

In the cases just described, the cell *wall* loses water, causing the difference in tension and the resulting motion or curvature. In the sporangia of the ferns the mechanism is somewhat different. When the cells of the annulus dry out, the walls remain saturated with water as the water moves out from the lumina to replace that lost by evaporation. The watery contents adhere to the wall, but at the same time the cohesion of the water molecules for each other is also very great (Chap. XXI). As the water content decreases, the cell wall thus becomes deformed and the thin outer walls of the annulus cells are pushed in toward the opposite sides, which are much thicker (Fig. 27). The entire annulus is thus shortened, with the result that it ruptures at the side where the cells are weakest. The surface tension forces draw the annulus slowly backward, when the water menisci in the cells suddenly break, with the result that the top is thrown back into place, violently catapulting the spores (Conard, 1908). In some ferns, this may later be repeated. If water is added, the spo-

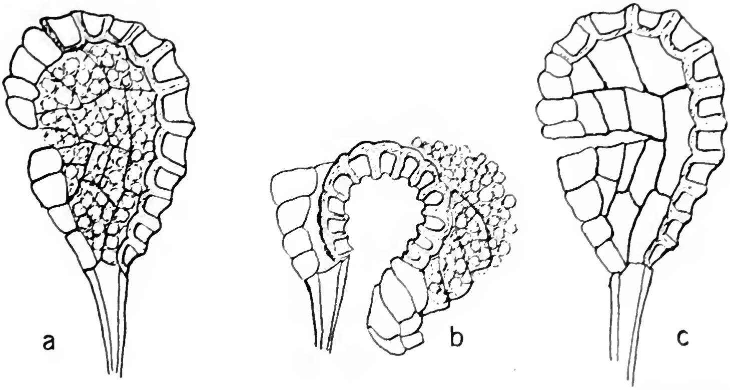

FIG. 27.—Opening of fern sporangium due to imbibition. a, sporangium starting to open; b, with annulus bent back scattering the spores; c, sprung back to the normal position but empty.

rangium closes and the cells resume their former position. The walls of anthers and the sporangia of other Pteridophytes behave in a similar manner, usually without sudden movements.

Turgor Movements.—Turgor movements are due to changes in the water content of the cells without any increase in number of cells and unaccompanied by any other growth changes. Such movements are produced (1) by *external* causes which can be modified at the pleasure of the investigator, or (2) by *internal* conditions of unknown cause. The former are called *paratonic* and the latter *autonomic.*

Autonomic movements due to turgor pressure are comparatively rare and are confined almost entirely to leaves or their modifications. In the Leguminosæ, Oxalidaceæ, and a few other families, the leaf base is swollen to form a parenchymatous pulvinus as described for *Mimosa* (Chap. XXVIII). Variations in the turgor on various sides of this pulvinus cause the leaf to describe a circle or ellipse. While this movement is not rapid and requires special apparatus for its observation in the majority of cases, in the classic case of *Desmodium gyrans* the movement can be seen with the unaided eye. Here the tips of the leaflets move in an elongated ellipse with a path several millimeters long. The movement is sometimes uniform but more often intermittent or jerky, from which the common name of "telegraph plant" has been derived. Under room temperatures the entire traject is covered in 3–5 minutes, but at higher temperatures the speed is increased so that the entire course may be completed in a half minute. Similar movements are displayed by the leaves of *Eleiotis bahave.* The advantage of such movements to the plant is unknown.

In addition to these rotating movements or *nutations,* leaflets undergo in some cases an up and down motion in one plane. In the common red clover (*Trifolium*), the leaflets slowly rise and fall, completing an entire traject in one to four hours. This movement in one plane, produced by changes in turgor of the two sides at the base of a dorsiventral organ such as a leaf or petal, is known as a *nastic* curvature. In nutations, the organ moves in more than one plane, while in nastic curvatures the movement is in only one plane, owing to the fact that the structure of the organ prohibits movements in other directions.

Of the paratonic nastic movements due to changes in turgor, two have been especially studied,—those produced by light and those by temperature. The movements due to light are commonly called "sleep," nyctitropic, photeolic, and photonastic movements. The dandelion (*Taraxacum*) and many composite flower heads open during the day and close at night; night-flowering plants such as *Silene noctiflora*, the evening primroses, etc., behave in the opposite fashion. This arrangement keeps the flower open when its pollinating insects are about and protects them against rain, dew, or wind at other times.

While the turgor changes are here seen to be of advantage, it is harder to explain the movements of many legume leaves which drop at night and fold together. In this night or "sleep" position, as seen in the clover and oxalis, the petiole curves either up or down and the blades are generally placed with one definite face outwards. The night position is often such that the stomatal surface is directed inwards, and it has been suggested that this arrangement serves to check radiation and the formation of dew in the stomata, thus favoring transpiration in the morning. This explanation is based upon the assumption that transpiration is an advantage, which is very doubtful (Chap. XX). If these movements have any advantage, which is by no means certain, it would probably be in checking transpiration during the night and thus increasing the turgor for the following day's work. Since the changes in light are the most pronounced at dawn and twilight, the photeolic movements are easily observed at that time. In *Oxalis* the temperature seems to play a rôle but humidity is of little importance; while in the legume leaflets, humidity is important (Gates, 1916). Such movements, produced by light and humidity combined, have been called *xerophotic*. Zimmerman (1929) has noted turgor differences of seven atmospheres between the upper and lower sides of the leaf joint (pulvinus) in such leaves.

Thermonasty is shown by the perianth segments of many flowers, especially in members of the Liliaceæ (*Crocus*, *Tulipa*, *Ornithogalum*, and *Colchicum*), whose petals, during a rise of temperature, suddenly grow larger on the inner side of the base. The result is the opening of the flower. Crocus and tulip when brought in from out of doors to a temperature 15° C. warmer will open in three minutes. The crocus and *Gentiana ciliata* are sensitive to a difference of 0.2–0.5° while the tulip, only slightly less sensitive, responds to a change of 1–3° C. When the temperature is lowered, the flowers close, which indicates that this growth movement is reversible and due to changes in turgor.

Turgor changes produced by shock with consequent movement have been discussed in the case of *Mimosa*. Similar movements resulting from the loss of turgor are to be seen in the barberry (*Berberis*), the stamens of which fly upward and inward close to the stigma if touched at the base during the pollen-bearing season. In some of the Cynareæ (Compositæ), the filaments contract when touched, and, as they slide down over the style, the pollen is scraped out. In still other plants (*Tecoma stans*, *Diplacus glutinosus*, and *Crescentia cujete*) the stigmas are sensitive and close when touched, as described by Newcombe (1924). Another example of note is Venus's flytrap (*Dionæa*) which bears trigger hairs on the leaves. If these are touched by an insect, the two halves of the leaf lock together quickly thus capturing the insect. Here the change in turgor occurs in the midrib of the leaf, where the osmotic pressure increases when the leaf closes. This growth is permanent and the leaf remains closed until the cells on the upper side grow and open the leaf again (Brown, 1916). In *Drosera*, on the other hand, during bending the cells on the concave side of the tentacles *decrease* in osmotic pressure, while those on the convex side remain the same (Hooker). These shock movements are sometimes called *seismonastic.*

It is often hard to separate autonomic from paratonic movements. In the case of *Desmodium*, it has been mentioned that the rate is affected by temperature. To that extent, the movement is paratonic even though the initial cause is unknown. The term "autonomic" is merely a word to cover our ignorance. Similarly it is hard to distinguish growth movements from turgor movements for reasons which are obvious to the careful reader. Nevertheless, for purposes of classification, the attempt to distinguish between these various types of movements has been made. The student must always remember, however, that all classifications are man-made, as helps to the memory and nothing more.

Growth Movements.—The movements due to *permanent* growth are curvatures which are produced by unequal growth. One side grows more than the other with the result that bending takes place. As in the case of the turgor movements, some cases are found in which the causes are as yet unknown; the regulating factors are *internal* and therefore difficult to determine exactly.

Among these autonomic growth movements are the nutations discovered and described by Darwin. The tips of many stems do not grow the same on all sides or at the same rate, with the result that the tip nods successively to all points of the compass describing a circle or an ascending spiral as the stem grows in length. In the case of twining stems, these nutations are very striking.

Nastic movements of an autonomic nature are seen in the opening of buds. When the primordia have reached the proper stage of development, the upper half of the bud scales grows more rapidly than the lower side and the scales turn back. Similarly at the opening of the flower, the upper half of the growing base of the petals and sepals grows more rapidly than the lower side and the flower opens. In some cases, as mentioned above, these changes are reversible and are connected chiefly with variations in turgor.

Paratonic Growth Movements.—These movements include the *tropisms* which differ from the nastic movements in two particulars. In the tropisms the direction of movement and growth depends upon the *direction* of the stimulus, while nastic movements depend upon changes in *intensity* rather than in direction. Secondly, tropisms occur chiefly in organs which are radially symmetrical and free to move in all directions, while nastic movements are found only in parts which, owing to their structure, are free to move in only one plane. Nastic movements are found consequently chiefly in leaves, petals, etc., where they occur in a vertical plane. Tropisms, however, occur in any plane, and even organs like leaves, which are not radially symmetrical, may exhibit tropistic movements.

Phototropism.—Of all the external factors which influence the growth and development of plants, light is one of the most important, as has been emphasized already many times; and in view of this previous work, the value of the responses here described will not be difficult to understand.

Stems are generally positively phototropic, i. e., they grow toward the light. The growth on the side nearest the light is checked, with the result that bending occurs. In a few cases (see below) the stems are negatively phototropic, but such cases are rare.

As in geotropism, there is a perceptive region at the tip of the stem, but it is larger and less localized than the perceptive region for geotropic stimuli. Just what the plant perceives is also an open question. Some have believed that the *inequality of illumination* is what is perceived while others have held that the *direction* was the important effective element. Still others have suggested that the plant is able to perceive the difference in *pressure* on the two sides of the stem. Since this difference in light pressure is almost infinitesimal, it would seem to be out of the question, and nothing is to be gained by the addition of metaphysical speculations.

The relation of hormones to growth has also been discussed (Chap. XVII). Not only is the downward flow of growth hormones checked on the illuminated side with the result that this side grows less, but this result is also favored by the fact that the permeability of the illuminated side seems to be decreased somewhat by the light (Blaauw). The result is that (1) the cells on that side are less turgid and (2) the growth-inhibiting hormone can diffuse into these cells more easily. The checking of growth on the illuminated side and its promotion on the opposite side are thus seen to be decidedly complex phenomena.

During the development of the plant, the sensitiveness to light may change. The flower stalks of Kenilworth ivy (*Linaria*) are at first positively phototropic, but later, when the seeds have ripened, the phototropism becomes negative, pushing the fruits into crevices where the seeds can germinate. In other cases a plant may be positively heliotropic in weak light and negatively so in strong light. In the oat plant (*Avena*) such variations with intensity are very pronounced.

The amount of time required to cause bending varies inversely with the intensity of the light. Thus a light of 26,500 candle power required only 0.001 of a second, while light of 0.000439 candle power required 13 hours to bring about the same degree of curvature. Violet light is most effective in causing phototropic bending and yellow light the least, with the red intermediate (Fig. 28). Similar results were obtained with *Pilobolus*, with which it was also noted that the presentation time multiplied by the square root of the wave frequency is nearly a constant. Sensitiveness generally diminishes with age and also varies with the temperature and other external conditions.

Roots are negatively phototropic, but the direction of their growth is determined more by gravity than by light. Leaves are diaphototropic and set themselves at an angle to the incident rays, where they are in a position for maximum photosynthesis. On the edge of the crown of a tree, it is interesting to observe how the leaves are arranged to get the maximum of light with the minimum of shading. On the sides of buildings and walls the leaves overlap so little that they form what are called leaf *mosaics.* If the light is too intense, the position may be shifted, and in plants with the leaves in a vertical plane, they are so arranged that they get the moderate light of the forenoon and afternoon without being exposed to the intense light of midday. The edges of the leaves are thus directed to the north and south, and such plants as the wild lettuce (*Lactuca scariola*) and *Silphium laciniatum* have been given the name of "compass plants." That this adjustment is actually dependent upon the light intensity is shown by the fact that when the lettuce is grown in the shade, the north and south position is not assumed.

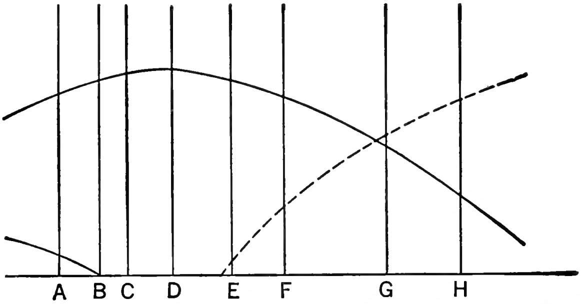

Fig. 28.—The rate of *growth* and *phototropic sensitiveness* of plants in various parts of the spectrum. The letters at the bottom indicate the Frauenhofer lines. The solid line is the curve of *growth* of sunflower seedlings. The dotted lines represent the *phototropic sensitiveness* of water cress seedlings. In what part of the spectrum is growth the fastest? In what part does phototropism occur most quickly? (After Palladin.)

Some flower heads are also positively phototropic. Species of *Helianthus, Hieracium,* and *Tragopogon* stand erect at night, but before sunrise the stems bend over towards the east. As the sun rises the heads open and then change their position during the day as the sun passes across the sky, so that in the evening they face the west. At sunset the stems become erect again, remaining in this position until morning. From this behavior the common name of "sunflowers" has been given to such forms.

Even some fungi show phototropism. The most conspicuous example is that of the coprophilous fungus, *Pilobolus crystallinus*, which grows on cow or horse dung and produces numerous fruiting bodies called sporangiophores. If these plants are grown in a darkened chamber with a small window at one side, the sporangiophores all point toward it, and when the sporangia are ripe, they are shot away from the plant directly towards the light. The sporangia stick to the pane of glass, showing by their location with what fine precision they were aimed.

Other Tropisms.—*Geotropism*, or the movement in response to gravity, has been discussed in the last chapter. *Chemotropism* (turning in relation to chemicals) is of very common occurrence. Fungi are negatively chemotropic to their own metabolic products. Root hairs are very sensitive to nutrient elements and to toxic substances, going towards the one and away from the other. Pollen tubes are very sensitive to sugars and grow in their direction. It seems probable that the growth of the tubes down through the style is determined largely by chemical compounds found in the pistil and ovules. *Hydrotropism* (turning in relation to water) is a form of chemotropism, seen especially in roots and root hairs, which are positively hydrotropic. *Aërotropism*, which is important in the case of roots growing in swampy places and in the growth of pollen tubes, is another form of chemotropism. The former are positively aërotropic, while the latter are negatively so and grow away from the air towards the interior of the style, where oxygen is less abundant. Fungi are very sensitive to many varieties of materials and serve as excellent subjects for the study of the various kinds of chemotropism, including hydrotropism and aërotropism.

Thermotropism is of slight importance in nature. Plant parts require an optimum temperature and will grow in the direction of a source of heat which is nearer the optimum than the one to which they are subjected; if the temperature is not as favorable, they will grow away from the source of heat.

When roots are grown in a current of water of a moderate velocity, they set themselves against the current. The precise nature of the response is not known, but it seems to be due to the pressure of the water, which sets up resisting or counteracting strains in the plant. This reaction, which is known as *rheotropism*, has little importance and, like *electrotropism* (growth in response to electricity) or galvanotropism (turning in response to an electrolytic current), has little significance in nature. If the ions are responsible for the plant movements seen in an electrolytic solution, then galvanotropism may also be a form of chemotropism.

Of more importance is *thigmotropism* or *haptotropism*, which is irregular growth produced by contact, friction, etc. This is of especial importance in twining plants and those with tendrils. The side in contact with the support grows less than the opposite side, and twining results. Tendrils often have tactile spots which are exceptionally sensitive. The initial bending may be produced by a change in turgor, which is followed by differences in growth. The twining is thus a result of autonomic nutations, of turgor changes, and of permanent growth changes, stimulated by the friction of the support. Since light and gravity also play a part, it is seen that the twining of stems and tendrils is a very complicated phenomenon.

Traumatropism, or the differential growth due to wounding, has already been discussed (Chap. XXVII). These many citations to other chapters should serve to impress upon the student the fact that the phenomena of living matter are closely interrelated. It is not possible to discuss growth without considering movement and irritability or, *vice versa*, to consider movement apart from growth. All of these coördinated and related processes make up the phases of the unexplained mystery called life.

QUESTIONS

1. Why are plants less motile than animals?
2. What are cœnocytes, fern prothallia, rhizomes, flagella?
3. Describe the movement of *Oscillatoria*.
4. Distinguish between phototaxy, photonasty, and phototropism.
5. What are floral clocks?
6. What is the derivation of the words thigmotaxy, autonomic, paratonic, traumatropism, and rheotropism?
7. Give two reasons why leaves in the shade are generally greener than those in the bright sunlight.
8. "The presentation time multiplied by the square root of the wave frequency is nearly a constant." Explain clearly.
9. Guttenberg (1924) found that when *Avena* and *Coleus* were illuminated by oblique light, the response was proportional to the sine of the angle made by the beam with the plane of the illuminated surface. Is this in agreement with the results discussed in the previous chapter in connection with geotropism and the laws of acting stimuli?
10. Which is the better word for the movements of oxalis leaflets—nyctitropic or photeolic? Why?

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CHAPTER XXX

REPRODUCTION

> Pray tell us from whence this impertinence springs,
> The sexes at once to confound.
>
> —WHITEHEAD.

Organisms, in general, sooner or later die. The length of life varies with different species and individuals, but after some months or years (in some cases even hours or days) external or internal conditions become unfavorable for life and the existence of the individual, as such, ends. If a species is not to become extinct, some provision must be made for reproduction or the formation of new individuals, since only those organisms which have this power can survive in the struggle for existence.

Vegetation and Reproduction.—All useful structures and responses have one of two purposes; they serve either (1) to keep alive the individual organism which possesses them or (2) to keep alive the race (species). The former structures are called the *vegetative* ones and the latter the *reproductive.* As ordinarily catalogued, the root, stem, and leaf comprise the vegetative organs, while the flower, fruit, and seed are the reproductive structures.

The question sometimes arises as to which of these two functions is the more important,—vegetation or reproduction,—the life of the individual or the life of the race. The answer depends simply upon the point of view. To the individual, his own life is the more important, but if we judge by the facts of human experience, from the point of view of nature (assuming that nature has a point of view) the life of the race is much the more important. Individuals rise and fall, wars between individuals and species with their millions of slaughtered dead come and go, but the race continues. Nature seems to care little about individuals; with her it is the species that counts.

Nature of Reproduction.—Reproduction commonly implies (1) separation from the parent plant, (2) rejuvenation, and (3) multiplication. The young which result from reproduction lead a life distinct from the parent and start out with rejuvenated protoplasm. While multiplication is often one of the most striking characteristics of reproduction, it is not an essential one. A poplar tree may produce annually twenty-five million seeds, of which a few survive. The fungi produce millions of spores, all capable of forming new plants if placed under a favorable environment. In these cases, there is a pronounced multiplication of individuals, but in many cases the number of new individuals produced is not sufficient to replace the two parents when they die.

Although cytology and comparative morphology have taken over the fields of botany which deal with the formation of the sex cells and the way in which various plants have solved the problem of reproduction, a brief résumé of the physiological aspects of the subject is here included for those not familiar with these fields.

Asexual Reproduction.—Reproduction is of two types depending upon the number of parents required to complete the process and to form the new individuals. In *asexual* reproduction, only one parent is needed. No special sex cells are formed and, for these reasons, this type of reproduction is also called *vegetative, agamic,* and *monogenetic.* Although this is the simplest type of reproduction and is the only kind found among the earliest of plants such as the bacteria and the blue-green algæ, in which the mature cell when ready to reproduce simply splits in two forming two cells where one was before, it also occurs in the highest plants along with sexual reproduction. This simplest type of asexual reproduction, called *fission,* also is found among the lowest animals.

Many of the simpler plants form special reproductive bodies which are usually one-celled and capable of resisting dry weather, cold, etc., for some time. These bodies, called *spores,* are formed in great numbers by the Fungi, Bryophytes, and Pteridophytes.

In addition to spores, the Bryophytes (Liverworts) develop specialized *groups* of cells called *gemmæ* which are capable of reproducing the entire thallus of the plant. Similarly in the seed plants, many forms have special asexual methods of reproduction in which some vegetative tissue is especially fitted for reproducing the plant. Among these may be mentioned the *bulbs* of the onion, the *tubers* of the potato and artichoke, the *corms* of Jack-in-the-pulpit (*Arisæma*), the *runners* of the strawberry (*Fragaria*), etc. In fact all the "reproductive stems" are examples of asexual reproduction. Roots also may serve this same purpose, as in the dahlia and sweet potato. Many of our most troublesome weeds, like the Bermuda grass (*Cynodon*) and quack grass (*Agropyron*), have extremely efficient asexual methods of reproduction.

Some plants which normally do not reproduce asexually, may be so propagated by means of "cuttings," which are merely pieces of stems that are removed and stimulated to produce adventitious roots, thus establishing connection with the soil. Many shrubs, trees, and herbaceous plants are propagated commercially in this way, including the grape, banana, rose, and begonia; sweet potatoes are propagated entirely in this fashion. Some workers have insisted that vegetative reproduction continued for long periods of time results in deterioration and have cited the potato, which frequently does deteriorate with age, as proof of their contention; but it has recently been shown that this deterioration in the potato is produced by filterable virus diseases and does not occur in strains lacking the virus. Furthermore, the observations of plants known to have been propagated asexually for even thousands of years indicates that there is no inherent defect in the method as such. The yam (*Dioscorea*) has been grown vegetatively in China for over 2,000 years, and there is no evidence that it is deteriorating. The sweet potato has lost the power of sexual reproduction in temperate climates and has been cultivated for many generations without apparently losing its vigor. Similarly the fig, date, and grape have been grown vegetatively for centuries without showing any signs of becoming decadent; and the saffron crocus (*C. sativus*), which is sterile, has been grown from the asexual, lateral corms since the Middle Minoan period (1900–1800 B.C.), showing that it is possible to reproduce a plant asexually without any visible deterioration for nearly 4,000 years.

Grafting is a form of asexual reproduction by cuttings where the cutting or *scion* is caused to grow in the stem or *stock* of another plant instead of in the ground. Although plants as unrelated as the cabbage and tomato have been successfully grafted together (Daniel), for the practical success of this operation the two plants which are grafted together must be of congenial relationship, i. e., they must not be too unrelated taxonomically. One can graft apples and pears together as well as tomatoes and potatoes, but one cannot graft successfully apples and cherries, which, it is interesting to note, give negative results also with the precipitin test (Chap. XIV). Furthermore, plants must be taken which have lateral meristem (cambium), since it is this region which grows together at the cut surface and ultimately causes the joined surfaces to knit into one connected whole; and it is well to get as much cambium together as possible. From the results of grafting some very interesting physiological data have been obtained. Thus Vöchting (1894) and Daniel (1921) have shown that when the sunflower (*Helianthus annuus*), which produces starch as the chief reserve carbohydrate, is grafted to a stock of the artichoke (*H. tuberosus*), which produces inulin, each plant continues the production of its own characteristic reserve regardless of the kind of material furnished it from the leaves above. The sunflower at the top may form starch and send down sugar to the roots, but in the artichoke at the base this is stored up as inulin. The same individuality is shown when the graft is reversed.

Sexual Reproduction.—In *sexual* reproduction, the union of two sex cells called *gametes* is required. This type of reproduction, which requires a sexual union of two cells, is also called *digenetic*. The development of sexual reproduction has not occurred suddenly in the evolution of plants but has evolved by gradual steps. In the blue-green algæ all reproduction is asexual. Sexual reproduction first appears with the green algæ in forms like *Spirogyra*, where the gametes are not special cells but are transformed vegetative cells that unite in pairs to form the zygospores. The gametes look alike (although York thinks the male filaments are less vigorous and contain less starch than the female) but behave slightly differently. One (the female) remains passive in its cell while the other (the male) moves through the conjugation tube into it.

In the black mold (*Rhizopus nigricans*) the gametes look alike and behave alike, meeting in the middle of the conjugation tube; but Blakeslee has shown that the plants from which they come are either male or female strains, and before conjugation can result, it is necessary to bring together plants of opposite strains or sexes.

In *Spirogyra* the gametes are transformed vegetative cells and in *Rhizopus* they are single cells cut off from the vegetative hyphæ. However, in *Vaucheria*, another green alga, *sex organs* which contain the gametes, make their appearance. In this genus, the sex organs are on the same plant, as is true in most of the Thallophytes, but in many Bryophytes like *Marchantia* the sex organs are on different plants, one of which bears a male sex organ with sperms (male gametes) and the other a female sex organ containing an egg (female gamete).

In the higher plants, where the sporophyte is the conspicuous generation, the sexual differences in the gametophytes have been thrust farther and farther back into the sporophyte generation. In the more advanced Pteridophytes, the spores which give rise to the male and female gametophytes become differentiated into *mega* (female) and *micro* (male) spores. Ultimately the leaves (*sporophylls*) which bear these spores become differentiated into mega- and micro-sporophylls, and it is in this situation we now find the seed plants with their pistils (megasporophylls) and stamens (microsporophylls). In most plants, the stamens and pistils are in the same flower, in which case the flower is said to be *perfect.* In other cases, as the pine, corn, oak, cucumber, and walnut, the stamens are in flowers on one part of the plant, while the pistils are in other flowers on the same plant. Such plants are said to be *monœcious.* In still other species (date palm, hop, willow, and poplar), the staminate and pistillate flowers are on different plants, which are then said to be *diœcious.* From the point of view of sex evolution, these plants are the most advanced, but relatively few plants have reached this degree of differentiation. Animals have gone through these same stages of evolution, but have made more progress, with the result that all of the higher animals have lost completely the power of asexual reproduction, and male and female gametes are produced by separate individuals. *Perfect* forms among the animals are said to be *hermaphroditic.*

Origin of Sex.—Why sexual differentiation should have evolved, why it had survival value, is a question that has been given much thought and speculation. Its chief advantage seems to have been that through the union of two germ plasms in sexual reproduction and the resulting combination of characters which results from crossing, there has been the possibility of greater variation, and nature has had more forms from which to select the survivors. There was a greater chance for happy combinations of characters, which would result in forms especially fitted to survive the manifold conditions of the world. There has thus been the greatest progress in those places where there was the greatest diversity of mixing germ plasms. Vegetative or asexual reproduction is a *quantitative* method which may result in the rapid multiplication of a species, but sexual reproduction is a *qualitative* one which, although in many cases not so successful from the point of view of numbers, has the great advantage of introducing new qualities in the form of new combinations of characters.

The advantage of cross-fertilization, which was first emphasized by Darwin, has the same reasons at its base. According to Darwin, nature "abhors self-fertilization" as she does a vacuum, and the many devices of plants to bring about cross-fertilization and to inhibit self-pollination and fertilization support this view. Among these devices are diœcism (see above), the ripening of stigmas and pollen at different times, etc. The complex relationships between insects and flowers, which result in cross-pollination, seem to have had a decided survival value. While all these examples show the "abhorrence of self-fertilization," some plants like *Viola*, *Polygala*, and *Alisma* possess flowers which never open and so must be self-pollinated. Such *cleistogamous* flowers insure pollination in case the cross-pollination by the opened flower fails. East and Jones (1919) have shown that inbreeding or close fertilization is quite all right and not to be deprecated in case the stock is a good one. In case it is bad, then inbreeding will result in the continuation and intensification of bad qualities; but if the organisms have no bad qualities which are dominant in crosses, then there is nothing to fear. In the development of fine herds of cattle, horses, and other animals, inbreeding is a very common practice with no visibly bad results. Since the physiology and morphology of sexual reproduction has been taken over by the science of genetics based upon the discoveries of Gregor Mendel, little more will be said upon this subject here.

Determination of Sex.—The cause of maleness and femaleness in organisms has always been a question of much popular as well as scientific interest, but only within this century has any progress been made in solving this attractive problem. In animals it has been observed that the males and females generally differ in their chromosomal constitution. It commonly happens that the male has an odd number of chromosomes in the cells of the body while the female has an even number. This body (somatic) number is twice that of the gametes so that when reduction occurs, at the time of the formation of the sex cells, the female gametes will be all alike with half the somatic number; while the male gametes are of two kinds, because an odd number of units cannot be divided into two equal halves. If the male gamete with an even number of chromosomes unites with an egg cell, the result is a female, while if an odd-numbered one fertilizes the egg, the result will be odd-numbered and a male. In *Protentor* (a bug), the somatic number of chromosomes is thought to be 28 for the female and 27 for the male. There will thus be produced egg cells with 14 chromosomes each and sperms with 14 and 13 chromosomes. If a sperm with 14 chromosomes unites with an egg, the result will be a female with 28 chromosomes, while if a sperm of 13 chromosomes fertilizes the egg, the zygote will develop into a male.

In plants, sex is not so distinct as in animals. Most plants produce bisexual flowers with both kinds of gametes. Only a comparatively small number of plants are diœcious, so that it would seem difficult to connect sex with chromosome numbers as in animals, but Allen (1917–1919) has found in the gametophytes of *Sphærocarpus*, one of the liverworts, that the thalli which give rise to archegonia (producing egg cells) have eight chromosomes, one of which is a large X-chromosome; while the thalli which produce antheridia and sperms have, instead of the large X-chromosome, a small Y-chromosome. The sporophyte contains, of course, both the X- and the Y-chromosomes, which are separated when the asexual spores are formed; those spores which obtain the X-chromosome develop into female thalli while those which contain the Y develop into male thalli. Miss Blackburn (1923) reports what seems to be chromosome sex differentiation in *Lychnis* and *Populus*, the female having an XX complex and the male an XY. There are thus produced two kinds of male gametes. In the comparatively few (about a dozen) genera where sex in plants is demonstrably connected with the germinal constitution, the homozygous condition is generally in the female and the heterozygous in the male.

While the chromosomes are connected with sex in the forms cited, it remains to be proved that the chromosomes *determine* sex. Are they the cause or merely an accompaniment? There is plenty of evidence to show that sex is determined not only by the germinal constitution but also by the environment. In the animal kingdom, the work of Riddle (1914–1917) is classic in this field of study. He has shown that in pigeons which lay two eggs to a clutch, the egg which is laid first develops into a male bird and the second egg into a female. The first egg has less stored food, a higher water content, and a higher metabolism than the second. As the season advances and the birds get more food, all the eggs have more stored food and the percentage of females increases. Furthermore, female birds which come from eggs early in the season often show secondary male characters which are lacking completely in birds from eggs hatched later in the season.

Goldschmidt (1916) has concluded from work on gypsy moths that between the male and the female numerous sex-intergrades occur and that the individuals of either sex may be made to develop the characters of the opposite sex, the degree of change depending upon the age at which the experiment is started.

In *Bonellia*, a worm, the male is small and degenerate, living parasitically upon the female. If the young are grown in an aquarium by themselves, they develop into females, but, as Baltzer (1914) has shown, if they are placed in with mature females, they settle upon them and develop into males. By varying the time they rest upon the female, all degrees of sex-intergrades may be obtained.

While in the human species, sex is connected with the chromosomes, the secondary sexual characters seem to be determined by other factors, and various intergrades between the sexes are not unknown. It is probable that even here masculinity and femininity are relative terms with intergradations, so that the 100% male or female is of doubtful existence. In war and famine the percentage of male children born is slightly higher than in times of peace and plenty, which may be the result of a lack of nutriment, thus connecting up the question of sex with nutrition; and Joyet-Lavergne has shown that the basal metabolism of males is 6–7% higher than in females. The "suffragette" type is often gaunt and slender; and the stage villain who traps the unwary country maiden is never a fat man.

In plants similar nutritional effects have been observed. If diœcious fern prothallia are grown under poor nutrient conditions, the relative number of antheridia is increased. Klebs, working with *Vaucheria repens*, which normally produces oögonia and antheridia in equal numbers, found that when subjected to a high temperature or reduced atmospheric pressure, the number of oögonia was reduced and the number of antheridia increased, with as many as five antheridia together and no oögonia. Similarly Tiedjens (1928) showed that increasing the amount of sunlight tended to decrease the relative number of female blossoms on cucumber plants; and Schaffner also found that in corn the sexuality of the tassels changes with the amount of light.

In diœcious plants such as the willow (*Salix*), hemp (*Cannabis*), and mulberry (*Morus*), the flowers are sometimes perfect, suggesting that both sex factors are present but only one is normally expressed; and *Salix* has been observed (Giessler, 1927) to change from staminate to pistillate. In *Myrica gale*, which is normally diœcious, all sorts of transitions are seen. Sometimes the same shoot will bear both male and female flowers, and sometimes a shoot will bear male flowers one year and female the next. When on the same shoot, those above tend to be female, indicating the presence of a nutritional factor. One of a pair of united male twins of *Arisæma triphyllum* was changed into a female by controlling the nutrition (Schaffner, 1926). Similarly Mækawa (1925) found that the corm of the Japanese *Arisæma* changes its sexuality from male to female as it grows older. However, after it has become a female, the sexuality can be reversed by growing in poor soil or by removing portions of the corm or leaves. One of the best examples of the *relative* nature of sexuality is seen in *Ectocarpus*, an alga. In this plant, the gametes may be grouped as male and female and these in turn can be graded, largely on the basis of size and activity, into gametes of varying intensity. Thus if gametes of the same sex but of very different "intensities" are brought together, the weaker one behaves as though of the opposite sex (Hartmann, 1931). Schaffner also reports that in some species of *Spirogyra* a filament may act as a male towards one filament and as a female towards another. The conclusion, therefore, is that sex is a *quantitative* and not a *qualitative* matter. The *tendency* for a particular sex is transmitted and inherited with the chromatin material of the gamete nuclei, but the *expression* of this tendency is modified by the conditions under which the zygote develops.

In view of this quantitative nature of sexuality in plants, one should not expect to find any sharply defined line of demarcation or reliable chemical tests for determining sex. In general, female plants, organs, spores, etc., show a lower intensity of oxidation and a higher reducing power than the male. Accordingly, the female cells are commonly richer in glutathione and poorer in catalase than their male counterparts, but there are many exceptions to this rule, as might be expected.

Development of the Embryo.—Before the embryo can develop within the seed, pollination and fertilization are necessary. These two processes must not be confused; the former is the transfer of pollen from the anthers to the stigma, while the latter is the actual union of the male and female gametes. The immediate primary effect of fertilization is the growth of the embryo plant. The effect of the pollen on the embryo is a question for the science of genetics, but there are certain secondary effects which are more within the province of the physiologist. Among horticulturists the idea is prevalent that the nature of the pollen determines the development of some of the fruit characters which lie outside the ovules, viz., color and form. Waite (1894) found that Bartlett pears show constant differences depending upon the kind of pollen used, and Lewis and Vincent (1909) showed that similar effects are to be observed in apples. Spitzenberg apples when pollinated by Arkansas Black are deep red in color as compared with the light red fruits obtained when the pollen is from Jonathan apples. Murneek (1926) found that, in the tomato, plants in which fertilization had taken place had a higher metabolic efficiency than those in which fertilization was prohibited, showing that stimulative effects may even extend into the vegetative organs. Swingle (1926) showed that in the date the size, shape, and color of the seed and the rate of development of the fruit varied with the type of pollen used. He suggested the term *metaxenia* for this direct effect of the pollen on the parts of the seed and fruit lying outside the embryo and endosperm. But it still remains to determine how many, if any, of the effects described by the previous workers (some of which are mentioned above) were truly metaxenic. In some cases they may have been due simply to an increased setting of seed, in which the difference in fruit size and shape might occur as a purely physical result. In such matters the experimenter must constantly be on his guard, since it is very easy to confuse results which *look* similar and to conclude that they are produced by similar causes.

Parthenocarpy.—In general, the failure of either pollination or fertilization results in the fall of the flower without the development of any fruit, but in certain cases the fruit may develop even though there are no fertile ovules to develop into seeds. The stimulus of the fertilized egg is consequently not indispensable for fruit development. Among the plants which are known to produce such fruits are the English forcing cucumber, some varieties of eggplant, Cellini and Charlamowski apples, Clairgeau pears, navel oranges, seedless grapes, and bananas. Although it is important to know in these cases whether the fruit development has occurred with or without pollination, such distinctions have up to the present time not been carefully made, and all these cases of fruit development without fertilization of the ovules (regardless of whether pollination has taken place) have been called *parthenocarpy*.

Fertilization.—The steps in fertilization itself seem to be largely chemical in nature. The attraction of the egg for the sperm is often purely chemical. The eggs or the sex organs secrete acids such as malic acid which exert a powerful influence over the sperms, although the reasons for this attraction may be something more than chemical. (From *ultimate* causes physiology is as far away as any of the sciences.) When the sperm reaches the egg, its chief function seems to be the initiation of a series of chemical reactions which result in the development of the embryo. Lillie has suggested that the sperm brings in a specific substance, "fertilizin," which starts these reactions. At any rate, fertilization by the sperm is not always necessary for development of the embryo. In all sections of the plant kingdom are to be found species which develop regularly in this fashion *without* fertilization, including *Chara crinita*, some varieties of apples, *Taraxacum* species, and *Antennaria*. This development of the egg cell without fertilization is called *parthenogenesis*, which in *Marsilia* may be obtained by subjecting the spores to high temperatures. In fact parthenogenesis is not uncommon in both the plant and animal kingdoms. It is the normal method of reproduction in many insects and rotifers, and Loeb devised means of stimulating artificially the eggs of animals to develop parthenogenetically where normal fertilization had been previously considered necessary. Even frog eggs were made to develop without the influence of any sperm, and in this way the famous "fatherless" frogs were obtained. Therefore, there is still hope for those female members of the human species who are struggling so hard for freedom from the slavery and shackles of man.

QUESTIONS

1. What is the survival value of sexual reproduction? of asexual?
2. What is the meaning of *homozygous* and *heterozygous?*
3. Distinguish between sex-determination and sex-regulation.
4. What is the derivation of *parthenocarpy*, *agamic*, *ovule*, and *parthenogenetic?*
5. What are the real sexual organs of a Spermatophyte?
6. Describe the sexual reproduction of *Spirogyra*, *Rhizopus*, and *Vaucheria.*
7. What are chimeras?
8. What is the Manoilov reaction and what is its purpose?
9. Trace the steps in the "reduction division" when gametes are formed.
10. According to Yasuda and others (1927) pollen germinates more readily in the stigmatic secretions from another flower than in those from the same flower. Of what value is this?
11. Male and female plants have been grafted together without influencing the sexual expression of either stock or scion. What may be concluded from this concerning sexuality in plants?
12. Distinguish between *xenia* and *metaxenia.*
13. What is the sex chromosome situation in man? Does it resemble that in *Protentor* or that in *Populus?*

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CHAPTER XXXI

REPRODUCTIVENESS AND SEED GERMINATION

> Say which grain will grow and which will not.
> —MACBETH.

Vegetativeness and Reproductiveness.—Why plants reproduce *when* they do and the conditions which determine whether a plant will develop vegetative structures (stems and leaves) or reproductive organs (flowers and fruits) has long been a matter of study. It was held for many years that there was something incompatible between vegetativeness and reproductiveness; the plant could not produce both shoots and fruits and must consequently be either vegetative or fruitful. It was also thought that the responses were more or less according to the needs of the plant. If conditions were favorable for the growth of the individuals, then the species was not in danger and reproduction would not occur. If, however, conditions became unfavorable for growth, it was "high time" to consider the species. The plants then "got busy" and provided for the perpetuation of the species before dying. These were the ideas in vogue until the work of Kraus and Kraybill (1918), confirming earlier hypotheses of Klebs, placed the matter upon a firmer basis. Working with tomatoes, they showed that the kind of growth depends upon the relation of the carbohydrates to the nitrogen compounds present in the plant. With neither nitrogen nor carbohydrates, the plant neither grew vegetatively nor reproduced. When the carbohydrates were not too scanty, an excess of nitrates over carbohydrates resulted in vegetative growth but no reproduction. As the carbohydrates increased in proportion to the nitrates, there was an increase in fruiting and a decrease in vegetative growth. The reproductive and the vegetative growth may thus be controlled at will, depending upon the *balance* between the nitrogen compounds and the carbohydrates.

These results agree with experience. Fertilizers rich in nitrates tend to increase vegetative growth and to check reproduction. The carbohydrates stored in the plant during the summer's growth do not result in the formation of flowers until the unfavorable conditions of the late summer check the intake of materials from the soil. This lowers the intake of nitrogen while the carbohydrates increase proportionally, with the result that reproductive organs are formed. This work has many practical results. A vigorously vegetative, unfruitful plant may be made fruitful by decreasing the nitrogen supply; but it should be noted that if nitrogen is added to a plant lacking it, the result will depend upon the carbohydrates present. If the carbohydrate content is low, addition of the nitrogen will only increase the vegetative growth and have little effect upon fruiting; while if there is a high carbohydrate content, fruiting will be fostered. This explains apparent anomalies such as that noted by Hooker in apples and other fruit trees, where the initiation of fruit buds is associated with high *carbohydrate* content, but the actual fruit development is associated with an increase of *nitrogen* in the fruit spurs. The C/N ratio also has been found important in Cryptogams (molds), where spore formation is favored by carbohydrates and vegetative growth of the mycelium by nitrogen compounds.

The nitrogen-carbohydrate balance may not only affect the formation of vegetative structures but may even determine what type of organ (root or shoot) is produced. Nitrogen, as shown by Reid (1926) and others tends to inhibit the formation of roots, while carbohydrates favor their development. Thus Starring found that in tomato cuttings only 0.05 cm. of growth was obtained with a low carbohydrate and high nitrogen content; while with high carbohydrate and low nitrogen content, growth per cutting was 19.7 cm. Also in willow cuttings, there is evidence that at least a part of the polarity observed (Chaps. XVII and XXVIII) is due to differences in the C/N ratio at the root and shoot ends of the cuttings. Roots appear where the ratio is high (least N and most C); shoots where it is low (Hicks, 1928).

Photoperiodism.—The length of the illumination period and intensity of the light also play an important rôle in the determination of vegetative and reproductive organs as shown by Garner and Allard (1920–1925), who grew several species and varieties of plants at various intensities of light and for various periods of exposure to light. In general, the vegetative growth of the plants varied with the length of the daily exposure to light (or *photoperiod*), as might be anticipated. The plants exposed to a

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short photoperiod were reduced in size and many were induced to blossom and fruit, while others were unaffected as far as their reproductiveness was concerned. The Biloxi soy bean blossomed in 110 days of 12 hours or in 27 days of 5 hours; and tobacco under similarly changing conditions blossomed in 157 and 58 days respectively. It was, therefore, concluded that for each species of plant there is a critical photoperiod essential for fruiting. Those of a short photoperiod, i. e., those which are induced to blossom by daily light periods shorter than 12 hours have been called "short-day" plants, while those that blossom sooner when exposed to a photoperiod longer than 12 hours each day are called "long-day" plants. It is, consequently, possible to induce or delay fruiting by exposing or failing to expose the plants to their critical photoperiod. Thus *Aster linariifolius*, a typical biennial, can be made to complete its life cycle in a few months, and annuals such as soy beans, golden rod, and oats can be made to behave like biennials. Tomatoes, lettuce, and radishes are typical "long-day" plants; peppers are "medium-day"; and *Salvia* and ragweed are "short-day" plants. Buckwheat, on the other hand, is an example of a relatively small group of plants whose reproductiveness is not appreciably affected by the photoperiod.

A very large literature has developed about this subject in the past few years without adding much to the fundamental principles established by Garner and Allard. Auchter and Harley (1924) showed that at time of flowering long- and short-day plants had a similar composition with a high C/N ratio. Zimmerman and Hitchcock (1929) found that long-day varieties of dahlias had fibrous roots (low carbohydrate content) and no accumulations of nitrates; while short-day varieties had fleshy roots (carbohydrate reserves) and larger accumulations of nitrates in the leaves and stems. The results of these various researches on the chemical composition of short- and long-day plants are not opposed to those of Kraus and Kraybill, but rather are supplementary and show the effect of light in determining the chemical changes within the plant,—changes which are certainly connected with the ratio of the carbohydrates to the other substances present. Since the light is of supreme importance in determining the amount of carbohydrates in the plant, it is not surprising that it should play a prominent part in the processes under discussion.

The Seed.—The seed is the chief organ of *dispersal* rather than of reproduction, in the proper sense of the term, but it may be considered briefly at this point. A reproductive organ is one which contains sex cells and, although the *ovule* contains a female gametophyte with an egg cell, the *seed* or *ripened* ovule contains no such reproductive cells. It contains, on the other hand, an embryo plant which it maintains during conditions unfavorable for growth and then supplies with food when the germination season arrives. Stamens and pistils have also been called reproductive organs but, for similar reasons, the term is a misnomer when applied to these structures. As the science of botany developed, it was once thought that the pollen grains were male sex cells and the ovules were eggs, but the results of morphological research have made such views absurd. Names, however, do not change as rapidly as ideas.

The dissemination of the seed is brought about by water, wind, animals, and mechanical contrivances of the fruit or seed. The coconut is especially equipped for long sea voyages over salt water, and nuts from the West Indies have been carried by the Gulf Stream as far as Norway without being impaired in their viability.

Wind-scattered seeds generally have hairy appendages as in the willow (*Salix*), milkweed (*Asclepias*), and dandelion (*Taraxacum*), or are equipped with wings as in the elm (*Ulmus*), maple (*Acer*), and linden (*Tilia*).

Animal distribution is commonly accomplished through the possession of edible and attractive fruits as in the apple, cherry, and strawberry, all of which contain seeds that are thrown away or are capable of resisting the juices of the digestive tract. Another method is by means of mucilaginous secretions or prongs which catch in the clothes and fur of passing animals. The mistletoe (*Phoradendron*) has sticky seeds, and the beggar-lice (*Bidens*) and puncture weed (*Tribulus*) are excellent examples of the latter type of dissemination. In this case, it is more often the fruit than the seed which is provided with the adhering projections.

Many types of mechanical contrivances are used by plants for scattering the seeds. In touch-me-not (*Impatiens*) a slight pressure on the ripened ovary wall causes the pod to snap open and scatter the seeds. In the melon (*Cucurbitaceæ*) are to be found some of the most interesting devices from an evolutionary point of view. Wild melons are climbing plants which support the small fruits by means of tendrils. When the fruit ripens, the tendril or "curl" behind the fruit dies and breaks releasing the fruit, which falls to the ground with a crash and scatters the seeds. The melons of cultivation are much too large to be supported by tendrils and the plants are therefore allowed to run prostrate on the ground, but the tendril behind the fruit still becomes brittle and dies when the melon ripens! Some of the lower plants, among which are *Pilobolus* and the ferns already described (Chap. XXIX), have mechanical devices which aid in the dispersal of the reproductive bodies and the spread of the species.

Seed Germination.—Before seeds will germinate they must have oxygen, water, the proper temperature, and embryos which have reached a suitable degree of maturity. Temperatures that are optimum for growth are generally favorable for seed germination. The warm medium not only favors the chemical reactions that go on within the seed, but also promotes such physical processes as imbibition. But germination may often be hastened by *alternating* the temperature. Thus *Cynodon dactylon, Poa compressa, Typha latifolia,* and *Berberis thunbergii* when kept for 6 hours at 35–40° and then for 18 hours at 10° C. germinated more rapidly than when kept constantly at the higher temperature (Morinaga, 1924). Coville explains the germination of many plants the following spring by the favoring effect of the low winter temperatures. *Cornus canadensis* seeds, for example, kept over winter in the greenhouse at a temperature above 55° F. did not germinate when placed in the ground for 12 months, but when chilled for 2 months at 35–40° F. germinated within a month. Similarly Munerati found that 81% of oats germinated right after harvest at 12–15° C. but only 3% germinate at 32–35° C.

Some seeds, e. g., the castor bean (*Ricinus*), are equipped with a spongy absorbing organ, the caruncle, which aids in the absorption of water. Parasitic plants, e. g., mistletoe, require for germination that the seeds remain in contact with the correct host which, in some way not completely understood, furnishes the proper stimulus for development.

Light also plays a part in the germination of many seeds. According to Mitchell (1926) about 60% of all seeds tested are favored by light, including the sundew (*Drosera*), tobacco, mullein (*Ver-*

bascum), *Rumex*, and mistletoe (*Phoradendron*). About 30% are hindered by light, including clover, jimson weed, *Phacelia tanacetifolium*, *Solanum lycopersicum*, and *Amaranthus atropurpurea*. The remaining 10% are neutral to light, but with increasing age sensitivity to light decreases and hence more are neutral (Niethammer, 1927). *Aquilegia atrata* seeds may lie in the dark for ten years without germinating, but when exposed to light will germinate in fifty days. In *Spiræa tomentosa*, 50% of the seeds germinated in the light after 41 days, while only 2% germinated in the dark in the same period. On the other hand, 32% of the seeds of *Polygonum biflorum* germinated after 146 days in the dark and only 13% in the light (Mitchell). The light effect seems to vary directly with the wave length and is also proportional to the quanta of energy acting on the seed contents (Kommerell, 1927).

The light effect, however, is not always so simple, as shown by the fact that its inhibiting effect may be neutralized by placing in a 0.1M mineral acid for a short time or in a solution of potassium nitrate. Also in some cases, e. g., *Chloris ciliata*, the temperature plays a part. Above 20° C. these seeds are favored by light and below that are hindered (Gassner). Similarly *Amaranthus caudatus* seeds at 15–20° C. germinate only in the dark, but at 43–45° germination occurs only in the light. Between these points (at about 30° C.) germination is equally good in light or darkness, but the inhibitory effect of light can be removed by various nitrates (Van den Bos, 1920).

In many cases the light inhibiting effect seems to be related to the oxygen pressure. Light inhibited seeds are not inhibited in many cases if the oxygen pressure is increased 4–5 times normal. Likewise, light favored seeds may be inhibited with increases in oxygen pressure; and light-indifferent seeds are also commonly oxygen-indifferent over a wide range (Böhmer, 1928).

Many experiments have been carried out in order to hasten the germination of seeds by the same substances used in forcing buds, bulbs, etc. (Chap. XXVI), and since germination is a phase of growth it is not surprising to learn that, in general, the same stimulants found suitable for hastening normal growth processes are also of value here.

Dormancy.—Failure to germinate when water, air, the proper temperature, and all favorable external conditions are supplied (*dormancy*) is generally due, as Crocker and his students have shown, to one of six causes:

1. The seed coats are too hard for the growing plant to break open, and they permit the entrance of water, air, etc., in only small amounts. In the soil, freezing and thawing, along with the action of soil organisms and the chemical action of the soil solutions, soften the coat, and the seed germinates. Among seeds of this class are the pigweed (*Amaranthus*), shepherd's-purse (*Capsella*), peppergrass (*Lepidium*), water plantain (*Alisma*), and blackberry (*Rubus*).

2. The seeds are impermeable to water. Such "hard" seeds, when placed in water, do not swell quickly but only after a long time, owing to the presence on the outside of a hard, varnishlike film, which is impermeable to water. Among the common seeds in this class are many legumes such as sweet clover, red clover, alfalfa, and lupine. Red clover seeds may remain alive and hard for several years when placed in water, but any treatment such as scarifying (scratching), breaking, or removing the coat will result in immediate germination; and if shaken with sharp sand, 90% will germinate at once. Treatment with acids and alkalies may have the same result in softening the coat. Sweet clover and alfalfa seeds (air dry) were made permeable without injury by freezing in liquid air (at − 190° C.) for several days (Busse, 1930); and Davis (1928) increased by 50% the germination of these same seeds by exposing them to an hydraulic pressure of 2,000 atmospheres at 20° C. and then drying before exposing to germinating conditions. In the soil, freezing and thawing, combined with the chemical action of the soil solutions, ultimately soften or break these seed coats and permit germination.

3. The seeds are impermeable to oxygen. One of the best examples of seeds of this type is the cocklebur (*Xanthium*). The fruit or bur contains two seeds with different permeabilities. Both seeds require oxygen but the upper one requires about 35% more than the lower. The result is that in nature the lower seed will germinate the following spring after the fruit is ripe, but the upper seed does not receive enough oxygen till after a year has passed. Both seeds will germinate at once if the seed coats are ruptured so that gases may enter freely. *Pisum sativum* and Johnson grass (*Sorghum halapense*) have seeds which behave

similarly and germinate readily once the testas are removed and oxygen is allowed to enter freely.

On the other hand, some seeds like those of *Typha* (cat-tail) germinate better under water than in air, and only when the oxygen pressure is reduced do they germinate well. These seeds showed 96% germination in four days when 40–80% of the air was replaced by hydrogen or nitrogen, while in ordinary air no seeds germinated in this period and only 4% after ten days. This favorable effect of reduced pressure disappears when the testas are broken. This characteristic has evidently had survival value for seeds which ordinarily germinate in water and in swamps, where the oxygen pressure is abnormally low.

4. The embryos are not mature. Such seeds have very small embryos when the seed is developed, after which the slow process of maturation may continue, in some cases, for many weeks. Seeds of this class occur over a wide range of families and include the *Ginkgo*, *Ilex* (holly), *Ranunculus ficaria* (buttercup), *Erythronium* (dogtooth violet), *Corydalis*, and many orchids.

5. The embryos require a period of "after ripening." Such seeds will not germinate even though the embryo has attained its full size and all the other obstacles mentioned above have been removed. In the apple (*Pyrus*), peach (*Prunus*), hawthorn (*Cratægus*), maple (*Acer*), basswood (*Tilia*), bitter-sweet (*Celastrus*), red cedar (*Juniperus*), and ragweed (*Ambrosia*), the embryo is neutral or alkaline in the mature seed, and dormancy persists until the reaction of the embryo is acid. The acidity is probably necessary to favor the activity of the enzymes concerned in germination, which may hence be hastened by treating the seeds with dilute acids. A low temperature (5° C.) is also favorable to the development of acidity. In nature these changes are brought about by freezing and thawing and contact with the soil.

6. Secondary dormancy has set in. After seeds have become ripe and are able to germinate, it may happen that this capacity is lost and the seeds must be ripened again. This is *secondary* dormancy, and has been observed in *Brassica alba* and *Ambrosia trifida*. This type of dormancy is related to the oxygen supply and seems to be caused by the fact that the impermeability of the surrounding membranes to the carbon dioxide produced inside and to the oxygen outside causes a reversal or a loss of the

activity of the seed and a return to the dormant state. Removing the membranes (testa or integuments) also removes the dormancy.

Viability of Seeds.—The viability or the length of time during which seeds retain their vitality and germinative power is extremely variable. Aside from the influence of external conditions, the specific differences are very great. Some kinds will keep their vitality for only a single season while others will germinate after many years. Under favorable conditions the following durations of viability have been found:

Seed	*Years*	*Seed*	*Years*
Alfalfa	8	Onion	5
Asparagus	5	Peas	3
Beans	3	Pumpkin	5
Cabbage	5	Radish	5
Cereals (wheat, oats, etc.)	8	Red clover	8
Clover	2	Rice	3
Corn	2	Squash	5
Cucumbers	10	Timothy	6
Geranium	10	Tomato	4
Lettuce	5		

These figures do not indicate that all the seeds have lost their viability at this time but only that the germination percentage is not high enough after this period to justify their use as seed. Certain seeds (e. g., *Geranium*) are known to have retained their vitality from 50 to 100 years. If one computes from the percentage curves the time it would take for every seed to lose its viability, some seeds would be found to retain their germinative power 150–200 years, but there is no authentic record of any kind of seed which has retained its vitality longer than this period.

Viable seeds of *Nelumbium nucifera* (Indian lotus) were reported by Ohga to have been found in peat layers at such a depth where they must have been buried for 300–400 years, but seeds from the British Museum would not germinate when 215 years old, and later it was decided that the deposits in which these seeds were found might be no older than 150–200 years (Ohga, 1927). The stories of seeds from Egyptian tombs and similar places which have germinated after the lapse of centuries, are absolutely without foundation.

The causes of the loss of seed viability are (1) degeneration of the enzymes, (2) accumulation of toxic metabolic materials as the result of the slow katabolism that has been taking place, (3) exhaustion of food reserves, and (4) denaturation of the colloids of the embryo. It is therefore desirable to keep seeds in storage under conditions where metabolism will continue at the slowest rate possible. Cool, dry places fit this requirement. The seeds of *Salix pierotii* generally lose their viability within a week, but when kept in an ice box over dilute sulphuric acid and occasionally aërated, 53% may germinate at the end of a year. Pollen also retains its viability longer when kept dry.

It would be advantageous if one could tell the viability of a seed sample without planting some of it and waiting for time to tell. The most popular of the various methods tried is the catalase test. Many researches have tended to show that the more viable a seed is, the more catalase activity it possesses. Although this test is at best a relative one, it has exceptions. In *Pinus strobus* the seed with the higher catalase activity germinated the poorest (Knight, 1931). Also it is possible to kill seeds without appreciably affecting their catalase activity. Davis (1925–1926) found that dead seeds when soaked in warm water lost their catalase activity very rapidly, so that a more successful test can be made by comparing the loss in activity after soaking. If a seed loses 0.9 of its catalase activity after soaking, its viability is low. This is, consequently, a good attempt to make an *absolute* test for a *relative* condition. The reducing activity of seeds has also been used as a criterion of their vitality, but this method has the same objections as the catalase test.

While reproduction is of the highest importance in the production of seeds and fruits, the foregoing chapter has attempted to touch only a few of the broader problems in their general physiological aspects, leaving to the agronomist, the horticulturist, and the geneticist the development of those phases most closely connected with their special problems.

QUESTIONS

1. Why do seeds fail to germinate in wet, muggy soil?
2. Where would you expect a higher percentage of blossoms in the summer,—in the tropics or the arctics? Why?
3. How does one compute the viability of seed from the percentage curve?
4. What is the effect of the size of the seed upon the size and growth of the resulting plant?
5. Transeau (1916) found that in *Spirogyra* the normal length of the vegetative cycle varied inversely with the surface area of the cells. How might Kraus and Kraybill explain this?

6. Low temperatures favor flowering and seed formation, while high temperatures favor vegetativeness in the celery (Thompson) and cabbage (Miller). How can this be reconciled with the work of Kraus and Kraybill?

7. Bakhuyzen (1924) reported that blooming of wheat occurs when the ratio of water to dry substance falls to a certain point. Can this be reconciled with the results of the work on the C/N ratio?

8. Chinese Imperial rice flowered in 72 days when exposed to 7 hours of light daily, but blossomed in 106 days of 12 hours daylight each. Is it a "short" or a "long" day plant?

9. Would you expect the germination of forest tree seed to be favored by light or by darkness? Why?

10. The temperature coefficient of absorption of water by *Xanthium* and *Pisum* seed and by the grains of *Zea mays* between 5 and 50° C. is about 1.5 (Shull). What are the common names of these plants and what does the statement mean?

11. Lumière reported that extracts from dead leaves hindered germination of seeds, and offered this as an explanation of the fact that many seeds would not germinate in the fall. Outline a simple method of testing this hypothesis.

12. Guillaumin (1923) found that seed would keep longer in the dark in a vacuum than in ordinary air in the light. Give reasons for this.

13. Would you expect plants which thrive and set seeds in high latitudes, e. g., oats, to be "long" or "short" day plants? Why?

14. Many plants from the temperate zone will not blossom when transplanted to the tropics. Can you suggest any reasons for this?

15. It was stated in the text that *Salix pierotii* seeds retained their viability longer when kept in the cold over sulphuric acid. What is the function of the sulphuric acid?

16. How does one test the catalase activity of a substance?

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CHAPTER XXXII

DEATH

> They were born for immortality.
> —WORDSWORTH.

Man's interest in the duration of life and in the death which ends it is probably greater than his interest in any other biological problem. From the beginning of human life upon the earth, the question of death has had much more than an academic interest for him. Why is he here but for a season and then hurried away? Among multitudes of others, Bernard Shaw has lamented the shortness of man's stay upon the earth, and in *Back to Methuselah* has interpreted man's failure to progress at the rate he should as the result of the fact that as soon as he gets a bit of experience, he must pass on and take this knowledge with him. New generations must begin again at the beginning, and, therefore, only by lengthening the span of human life can the most profit be derived from individual human progress; if we all lived to be several hundred years old, we would have some chance to use our experience and to realize, as it were, on the investment.

The best scientific book on the general subject of death in animals, is that of Pearl (1922) on the *Biology of Death*, and much of the material in this chapter dealing with *animals* is taken from this book. Pearl calls attention to the fact that man has tried to prolong his life in two ways. First, by means of the sciences and improvements in surgery, medicine, etc., he has increased the span of his physical life. Not only is the death rate decreasing, but the *average* length of life has been increased about fifteen years from the days of the Greeks and Romans. Secondly, by supernatural means and the aid of religions, man has sought to extend his mental life after the death of the physical body. Since science deals with physical phenomena, the second method of prolonging life lies outside its sphere, but it will be discussed briefly in the final chapter, which is concerned both with philosophical speculations and with scientific achievements.

Science has been asked to prove that there is or is not life after death. To prove the former is not within its *realm*, and to prove the latter is to prove a negative, which is not within its *power*.

Although man is interested primarily in the death of the human species, because of its direct bearing upon his own life, any facts about death either in the animal or plant worlds may be of importance in helping him to solve the problem; and, for this reason, it is valuable to review briefly some of the facts known concerning death.

Known Facts Concerning Death.—1. There is an enormous variation in the length of life of different species and groups of organisms. The following table expresses the range found within the organic world:

Group	*Age limit*
Protozoa and simpler plants including bacteria	24 hrs.–no limit
Insects	24 hrs.– 17 yrs.
Fish	? – 267 yrs.
Amphibians	? – 36 yrs.
Reptiles	? – 175 yrs.
Birds	9 yrs.– 118 yrs.
Mammals	1.5 yrs.– 100 yrs.
Trees (*Sequoia*)	? –3,000 yrs.

In trees, which live so much longer than animals, there is present much dead tissue. At the *growing* regions, plants have an indefinite form of growth, although the bulk of the tissue is dead. Owing to the lack of specialization of plant tissues as compared with those of animals, it is possible for only a small region to be alive and the entire organism, nevertheless, to be spoken of as "living." Unlike old Rover of the nursery rhyme, biennials and perennials do not "die all over." In deciduous species, new leaves are formed each year, and in those which are "evergreen," the leaves last, at the longest, only a few seasons before they are replaced. The wood dies in time and the old phloem is pushed off with the bark. MacDougal (1926) reported cactus cells which, he thinks, are over 100 years old, and later (1928) he and Brown reported living ray cells more than two centuries old in the heart wood of *Parkinsonia*. If the ray cells are that old, they are probably extremely exceptional in the plant kingdom; plant cells, in general, live for only a relatively few years. It is thus seen that the life of a spermatophyte is not exactly comparable with that of a mammal; the simple animals and plants are much more alike in their structure and in their longevity.

It will be noted from the table that whales and elephants (mammals) are not so long-lived as many have supposed. Records have not been carefully kept, but it is probable that man is the longest-lived of any of the mammals. Even in man, authentic records of centenarians are very few. As Young (1905) has pointed out, before one can be absolutely sure of an age record, he must have the documentary proof of the following critical points:

1. Date of birth.
2. Date of death.
3. Proof that the two records are of the same person.
4. The marriage date and any other records which aid in establishing identity; this is especially valuable in the case of women.

Young considers the third point the most important. It is very easy to confuse the birth and death records of two different people of the same name, and such confused records have often been used to substantiate the claims of excessive longevity. After examining nearly a million records, insurance tables, etc., he found only thirty authentic cases of people who had died when over 100 years old (not including the cases mentioned in the Bible). Of these, twenty-one were women and nine were men. The most extreme case of longevity on record was that of a person who attained the age of 110 years, 10.5 months. When this is compared with the records of the deaths in the United States for 1916, when 649 deaths over 100 years were recorded (317 white and 332 black), the necessity for the documentary proof required by Young becomes apparent. No records should be believed without the corresponding substantiating proof.

2. There is no relationship between average length of life of a species of animal and any other fact in its life history, structure, or physiology. It has been suggested by Weismann (1891) that the larger an animal the longer its life, but there are so many exceptions to this rule that it has no value whatever. Man is one of the longest-lived of the mammals and yet he is by no means among the largest.

In plants there is a kind of correlation between the size of the individual and the longevity, owing to the indefinite habit of growth possessed by plants. The annuals are herbaceous and seldom attain in the one growing season a very large size. The perennials, which include the shrubs and trees, are the longest-lived of plants and the largest, but the peculiar plant habit of local indefinite growth mentioned above may explain here the difference between plants and animals. Animals are alive "all over" while plants are not.

3. Natural death (as distinguished from accidental death) is preceded by structural and functional changes in the body known as *senescence.* In man these visible changes are the bent posture, which is the result of the bending and fusing of the vertebræ; the wrinkled visage, due to the loss of plasticity in the tissues; and the shuffling gait, which is caused by a failing motor coördination. These visible external signs of senescence are accompanied by internal changes within the cells, especially in the nervous tissue. As the cells age, the nucleus becomes smaller in proportion to the rest of the cell, fewer nucleoli appear in the nuclei, and the pigmentation of the cells increases. The following data from the work of Hodge (1894) show the change from a child at birth to an old man of ninety-two years:

Spinal ganglion cells	*Baby*	*Old man*
Volume of nucleus	100%	64%
Visible nucleoli	53	5
Deep pigmentation	0	67
Slight pigmentation	0	33

In the antennary lobe of the nervous system of the honeybee, changes have been found similar to those in man. The young cells contain large, round nuclei and small amounts of cytoplasm. In the older cells, the nuclei become stellate in shape, and only a small proportion of the cell is rich in protoplasm. While Minot (1908) considered these changes as the *cause* of senescence, it is more probable that they are accompanying results. However that may be, the correlation of the two phenomena,—senescence and cellular changes,—are very striking as expressed by Conklin (1913), who writes:

> By all odds the most important structural peculiarity of senescence is the increase of metaplasm or differentiation products at the expense of the general protoplasm. This change of general protoplasm into products of differentiation and metabolism is an essential feature of embryonic differentiation, and it continues in many types of cells until the entire cell is almost filled with such products. Since nuclei depend upon the general protoplasm for their growth, they also become small in such cells. If this process of the transformation of protoplasm into differentiation products continues long enough, it necessarily leads to the death of the cell, since the continued life of the cell depends upon the interaction between the general protoplasm and the nucleus. In cells laden with

products of differentiation, the power of regulation is first lost, then the power of division, and finally the power of assimilation; and this is normally followed by the senescence and death of the cells.

Benedict (1915) and Finardi (1925) found that in the wild grape (*Vitis vulpina*) and in many other woody perennials the meshes between the veins of the leaves get progressively smaller as the plants grow older. That this condition is propagated by cuttings is important because it shows the failure of asexual reproduction to produce rejuvenation in these cases. Benedict interpreted this decrease in the size of the vein-islets as due to a progressive decrease in the permeability of the cells. This requires that the cells be closer to the veins, with the result that "as the activities of life proceed, the cell is being gradually entombed by the inevitable decrease in the permeability of its protoplasm." This may explain also the increased dissection with age of the leaves of the dandelion (*Taraxacum*), as reported by Sears. Bergamaschi (1926) also found in grape, oleander, camellia, and other plants that the epidermal and cortical cells of the root decreased in size as the plants grew older. As plant cells die they also undergo many of the changes mentioned above: the cell walls become firmer and less permeable, the amount of protoplasm decreases in amount relative to the cell sap, and the nucleus gradually ceases to function. A large part of the increased electrical resistance that occurs with age is probably due, however, to the increased air space (Dixon). Belehradek (1927) found that the viscosity of plant protoplasm increases with age. It is interesting to note in this connection that the viability of seeds from old trees is frequently less than that from younger ones. Although Fabricius (1928) reported a slight increase of seed viability with age in conifers, his results are quite contrary to those of Hofmann (1921) and of Green (1920), who found that trees of *Pinus sylvestris* aged 15, 30, 45, 60, and 110 years gave seeds with a germination capacity of 87, 50, 30, 15, and 0% respectively.

Although the protoplasm degenerates as old age approaches, there are some who believe that this degeneration does not start in from birth. According to these observers, the protoplasm improves until the period preceding senescence, i. e., the curve which expresses the change of protoplasm with time, has a maximum point before senescence begins. This is in accord with the results of Boyagu (1919) who found that the respiration curve in the black locust (*Robinia*), the Scotch pine (*P. sylvestris*), the common privet (*Ligustrum vulgare*), and other plants, ascends to a maximum while the plant attains its full development, after which it decreases as old age sets in. Redfield believes that this improvement of the protoplasm in man with a maximum value between the ages of thirty and fifty explains the fact that men of genius are born when their parents are more than thirty-three years old. Although the opponents of this doctrine hold that the connection between genius and the age of the parents is one of environment rather than heredity, it is nevertheless a remarkable fact that a man has a much better chance of getting his name in *Who's Who* and of being what is called a "successful man" if his parents were over thirty years of age when he was born. Clute (1919) finds in the peony, daisy, and other plants that the doubling of flowers increases with age, which he interprets as an indication of better protoplasm. Although more doubling does occur with age and even though more geniuses are born of old parents than of young, it may be that the doubling of flowers and the flowering of genius are both signs of senescence rather than vigor as far as the *racial* protoplasm is concerned.

4. Natural death occurs normally and necessarily only in organisms composed of many cells. Woodruff has worked with the one-celled animal, *Paramecium*, which multiplies by simple fission, each animal at the time of reproduction simply splitting into two. There is no division into soma and germ cells here, but the entire body of the parent is divided between the two daughter cells, which grow and divide as before. Woodruff grew this form for about 12,000 generations, which in man would be nearly a half million years! In these 12,000 generations there was no sexual reproduction or rejuvenation by any sexual process. If *Paramecium* can live for 12,000 generations without the death of the material, it may be concluded that it is potentially immortal. As Pearl says:

> Now it is apparent that there is no place for death in a scheme of reproduction by simple fission such as this. Nothing is left at any stage to fulfill the proverbial scheme of "dust to dust and ashes to ashes." When an individual is through its single individual existence, it simply becomes two individuals who go merrily on playing the fascinating game of living here and now. There is, in short, no hope that messages will be received

from Paramœcia in the spirit world, for the simple reason that there is no chance for the soul of a Paramœcium,—assuming for the argument that he has one,—to get there.

In some of the flatworms (*Planaria*) vegetative or agamic reproduction takes place; the animal divides and each part continues its existence as a separate individual *ad æternitatem.* This same power of regeneration is also found in many other of the less specialized animals.

In plants the simple Thallophytes behave exactly like *Paramecium,* i. e., they divide by simple fission after which each half continues its existence quite independently of the other. In reproduction by means of cuttings, which in many of the horticultural forms like oranges, figs, and dates has gone on for centuries, the cells of the present plants are directly connected with those of their ancestors of many generations ago. Likewise plants which reproduce from rhizomes may grow indefinitely and for untold centuries. In such cases there has been no sexual reproduction, no separation into germ and soma plasma; and these organisms may also be said to be immortal.

Only in the Metazoa where there is a distinct division into germ and body cells do we find mortality. As specialization has progressed, certain cells (the germ cells) have been set apart to continue the reproductive work of the species. They are immortal, while the soma cells, connected with the vegetative work of maintaining the life of the individual rather than of the race, are mortal. Individuals and body cells are mortal; species and germ cells are immortal.

The soma cells, however, may be cultivated apart from the body in a nutrient medium for many generations. Carrell (1912) has a culture of the connective tissue from a chicken which he has been growing in a nutrient medium away from the chicken for several years. On January 17th, 1935, this connective tissue culture will be twenty-three years old, which is a far greater age than it would have reached if it had remained within the chicken, all of which goes to show that even the soma cells are *potentially* immortal. Lamprecht (1925), Börger (1926), Pfeiffer (1931), and White (1932) in a similar manner cultivated various plant cells for several weeks.

5. Life is a continuum. From the beginning of the time when life first appeared upon the earth it has gone on continuously.

The first organisms experienced no such thing as natural death, which has appeared in the course of evolution along with the increase in specialization. Death is not an inevitable and necessary accompaniment of life, but is the price paid for differentiation.

The Cause of Death.—With this summary of the known facts concerning death, it may be well to inquire into the reasons why, in differentiated forms, the soma cells should die. Though death is found only in differentiated cells, that does not explain why it is found even there. The following theories have been brought forward by various individuals to explain natural, physical death:

1. The theory of the Hebrews and of the Christian Church was that death is a penalty for the original sin. "The wages of sin is death." As a bare statement this has little scientific weight, although a careful analysis might show it to be in accord with the previous discussion. Differentiation is to many people a form of sin. Galileo, among others, was one who nearly died as a result of excessive differentiation.

2. Turning to the more seriously considered theories of death, that of Weismann (1881) may be mentioned. He proposed that death was an adaptation advantageous to the species, and was hence preserved by natural selection; it had survival value in the struggle for existence. This theory is now considered absurd and is cited as one of the worst examples of post-Darwinian logic.

3. Metchnikoff (1908) held that death was the result of intoxication arising from the absorption of putrefying products in the intestine. That this is not the cause of death, has been shown by rearing animals aseptically without preventing their death.

4. Jickeli (1902) held that death is due to the accumulation of poisons within the cells owing to the lack of complete metabolism; as time passes these accumulations increase until death ensues.

5. Verworn, Hertwig (1903), and Minot (1908) considered that death was due to the changes in the proportion of nucleus and cytoplasm in the body cells as described above. Such changes certainly do occur as an accompaniment of death, but whether they are the cause, or the result, is another question.

6. Conklin (1913) and Child (1915) have associated senescence and death with changes in the rate of metabolism. This theory is to be connected with the accumulations considered by Jickeli and the changes in the ratio of nucleus to cytoplasm as discussed

by Minot and others. Forms living in warm climates are not as long-lived as those in colder regions because of the higher rate of metabolism at the higher temperature. The human races of the tropics are not as long-lived as the races in cooler regions. The secret of long life is to "keep cool."

7. Lepeschkin, Dhar, and others have concluded that the chief factor in the death of cells is related to a denaturation of their protoplasmic colloids. This denaturation, associated with coagulation, may be due to various causes, but the end result is the same, viz., a failure of the cell to function normally, this decrease in normal function finally terminating in death.

8. Along with metabolic theories must be included that of Benedict, who associated death in plants with a decrease in permeability of the cells. This would result in the slowing up of the elimination of toxins and other injurious metabolic products, with their resultant accumulation in the cells. That such accumulations occur is certain, and the plant sheds the portions containing them when possible.

The fact that local death may occur in plants without general death is an accompaniment of the lack of complexity found in plants. If a plant possessed a nervous and a vascular system which connected all parts in the intimate way in which animal tissues are connected by the nerves and the blood vessels, local death would be impossible. Poisons formed in one place would be carried to another, and the death of one portion would result in the death of the entire organism. That such death does not result when leaves, the bark, the wood, etc., die, is a result of this inferior specialization, which thus has its advantages as well as its disadvantages.

9. Jennings (1912), Loeb (1919), and Pearl (1922) have concluded that death is the result of differentiation without the power to repair losses. As the germ and body cells have become differentiated, the latter have lost their embryonic nature and with it the power to repair all loss. They cannot remain juvenile like the germ cells, but must become old and die. This explains why plants with indefinite growth and several meristematic regions, have an indefinite length of life. Similarly, in the bacteria and Protozoa, the rejuvenated cells resulting from fission never lose the power to repair losses and hence never become senile. As Loeb states the case:

> All this points to the idea that death is not inherent in the individual cell but is only the fate of more complicated organisms in which different types of cells or tissues are dependent upon each other. In this case it seems to happen that one or certain types of cells produce a substance or substances which gradually become harmful to a vital organ like the respiratory center of the medulla, or that certain tissues consume or destroy substances which are needed for the life of some vital organ. The mischief of death of complex organisms may then be traced to the activity of a black sheep in the society of tissues and organs which constitute a complicated multicellular organism.

Plants and animals which are less differentiated are thus better life insurance risks than highly differentiated forms.

The question, then, as to *why* organisms die and why they die *when* they do, has been summed up by Pearl, who has shown that life is a continuum in which death is not inherent. Somatic death is the result of physicochemical changes in the cells of the organism. These changes result in organic disharmony ending in death, which is a new phenomenon appearing with differentiation and which occurs only in such soma cells as have lost the power of reproducing and rejuvenating themselves. The time of death is determined by the combined action of the environment and heredity. The length of time required for the injurious effects accompanying senescence and death to be completed, depends upon the external conditions and upon the inherited characters of the individual and species.

QUESTIONS

1. "There is no inherent reason for death." Explain this statement.
2. What are the chief factors that limit the age and size of a tree?
3. The Washington elm is now dead but close by on the Cambridge Common is a thrifty tree grown from a cutting. Has this tree any right to the veneration accorded the original elm?
4. What is an individual?
5. When is an organism dead?
6. What is *natural* death? Is this a good choice of terms? Explain.
7. Why is sour milk thought by the followers of Metchnikoff to favor longevity in man?
8. Since 1840 the average expectation of life in northern Europe and America has been prolonged from about 36 years to about 56 years. Is this an unmitigated blessing to society?
9. What and where is the oldest tree in the world? See the article by Chamberlain.

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CHAPTER XXXIII

MECHANISM AND VITALISM

> Myself when young did eagerly frequent
> Doctor and Saint and heard great argument
> About it and about; but evermore
> Came out by the same door wherein I went.
> —Omar Khayyam.

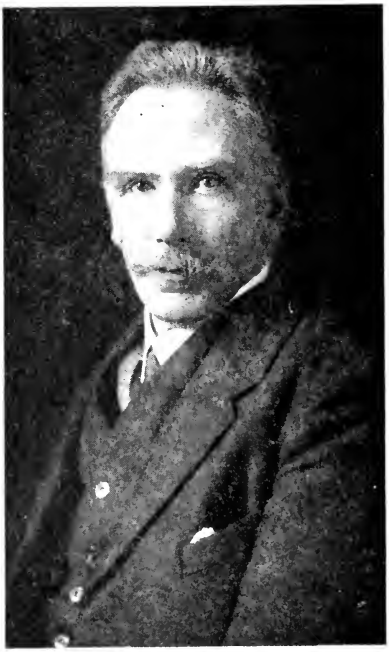
In the previous pages an attempt has been made to explain as many as possible of the life processes in the known terms of physics and chemistry. The question must have thus arisen in the reader's mind whether life is anything more than physics and chemistry. This brings us back for our last chapter to the discussion of questions of philosophy, which, we must remember, is after all the mother of science.

The Problem.—The question at issue is fairly clear-cut. The *vitalists* hold that life is more than mechanics and that there is a distinct gap between the organic and the inorganic worlds. This doctrine is known as *vitalism.* The *mechanists,* on the other hand, believe that all life phenomena may be explained by the physical and chemical constitution of living matter. This doctrine, known as *mechanism,* has been summed up by Pearl in his own statement of faith as follows: "Living things, whether single-celled or many-celled organisms, are essentially only physico-chemical machines of extraordinary complexity, but regardless of their degree of complexity only amenable to and activated in accordance with, physical and chemical laws and principles." The question stated briefly remains: Is life more than physics and chemistry? The vitalists maintain that it is and the mechanists that it is not.

From the beginning, the vitalists have been asked by the mechanists to describe or name some characteristic possessed by living things which was not equally the attribute of the inorganic or nonliving world. Up until 1828, it was thought that only organisms could make the compounds so characteristic of organic matter. In that year Wöhler synthesized urea, a distinctly animal product, and from that day to this, the synthesis of organic

Dr. Dennis R. Hoagland, Professor of Plant Nutrition, University of California, Berkeley. *Mineral nutrition of plants; Relation of plants to the soil.*

Dr. H. H. Dixon, Professor of Botany, Trinity College, Dublin, Ireland. *Transpiration and the ascent of sap.*



compounds in the laboratory has progressed by enormous strides. Even some of the products of the ductless glands (hormones), e. g., thyroxin, have been synthesized, so that this synthetic property can no longer be considered as forming a line of demarcation between the organic and the inorganic.

Disputed Points.—Other arguments have been put forward by the vitalists, and other qualities have been pointed out as distinctive of living matter. Among these may be mentioned the power of growth, reproduction, metabolism, irritability, autoregulation, and heredity, but to all of these the mechanists claim to have discovered analogies or homologies in the inorganic world. Growth, say the mechanists, is largely increase in size due to the addition of water. A dry, dead board grows by imbibition, and the swelling of a membrane filled with sugar solution, when thrown into a jar of water, is essentially the same thing as the growth of the cell. The addition of material or the increase of dry weight in organic growth is analogous to the growth of crystals, and differentiation may be seen in the formation of growth membranes produced by inorganic compounds when placed in contact with each other (Chap. XIX).

Reproduction in its essentials is the separation of a bit of protoplasm which is capable of living independently and which has the same properties as the parent from which it came. With the proper combination of chemicals and the help of surface tension changes, it is possible to get a drop to divide in two. The resultant drops possess the properties of the parent substance and, say the mechanists, thus show the *essence* of reproduction.

The characteristics of irritability are the delayed response and the expenditure of stored-up energy in making the response, but these are shown equally well by dynamite, by a motor, and in a thousand other ways. In setting off a stick of dynamite, the "impulse" is given when the match is struck and applied to the fuse. While the fuse is burning, the impulse or "stimulus" is traveling to the stick, and when the explosion occurs, this delayed response expends much more energy than it took to apply the match.

By autoregulation we mean the way in which the organism adjusts itself to changes in the external environment in order to keep the internal environment constant. The human body has a temperature of 98.6° F. Any increase in the external temperature results in increased evaporation from the surface which produces cooling. Any diminution below that temperature causes increased oxidation with a production of heat. Similarly, in maintaining the acidity of the blood, the regulating mechanism is so fine that an increase of one part of H^+ ion in 10^{14} parts of blood causes the respiratory rate to change in such a direction as to restore the normal condition. In plants the responses to gravity, light, etc., are examples of autoregulation (irritability); but the mechanists insist that autoregulatory systems much finer than those of organisms are to be found in the thermostat and other creations of man. Even in inorganic nature a kind of autoregulation may be seen in the bed of a river. As the amount of water rises, the width increases, with the result that the depth remains nearly constant as the stream fills the valley. Thus both the flow and depth are regulated.

Metabolism, from the mechanistic point of view, is purely mechanical (chemical). The burning of the sugar in the body is similar to oxidation in a flame. A candle is consequently a fine example of life to the mechanists as well as to the poets. The body of the candle is the stored food. The wick provides a means of translocating the digested (melted) food to the point where it can be oxidized in the flame to carbon dioxide and water, releasing energy in the form of heat and light. To be sure, the temperature is higher, but the action of enzymes which bring about oxidation at low temperatures is also purely chemical in nature.

As to heredity and its effects upon the organism, this is a point which was for a long time hard to duplicate in the inorganic world; but Van Bemmelen (1910) showed that even inorganic materials may vary with their past, i. e., they have a history, which is the *essence* of heredity. If a silicic acid gel is exposed to air containing various percentages of water vapor, the amount of water absorbed at a given tension of water vapor is not the same if the gel has been exposed to a lower tension as when it has been exposed to a higher tension (Fig. 29). If it has been in a drier atmosphere and the water vapor is now increasing, the curve of water absorption is expressed by A. But if it has been in a moister atmosphere, the amount absorbed is expressed by B. At a vapor tension of 6.3 mm. of mercury the water in the gel when the vapor tension is increasing is less than half what it is when the tension is decreasing. Consequently if one places such a gel in a vapor tension of 6.3 mm.

of mercury, the previous history of the gel can be told. This variation of a material with its past, which is the essence of heredity, is here shown by *inorganic* materials.

The vitalists have admitted in some of the above cases that the mechanists have defined the essence of an attribute correctly and have reproduced it in the inorganic world. They have thus, to some extent, been forced to retreat from their positions. In other cases, they have refused to concede these points. These matters are critical and are questions which every observer must decide for himself: (1) Have the mechanists defined correctly the essential character of the process? and (2) is their imitation of it a correct analogy?

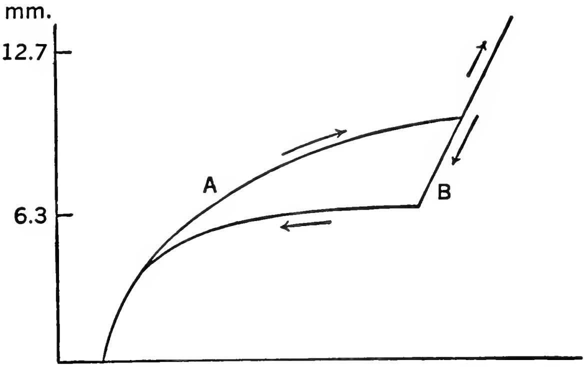


FIG. 29.—Water content of a silicic acid gel in equilibrium with varying tensions of water vapor. From the water content when the mercury vapor tension is 6.3 mm. one can tell whether the previous history of the gel has been one of an increasing or decreasing vapor tension. (After Van Bemmelen.)

Modern Mechanism.—Modern mechanism has led into two distinct fields,—biology and psychology. In biology its advance has been more or less gradual as more barriers have progressively yielded to physicochemical methods. In psychology, modern mechanism has led to *determinism*. If all acts are the result of the operation of physicochemical laws, then there is no freedom of the will and responsibility in the old sense must disappear. What seems to be free will or an exercise of discretion is merely an illusion. We think we are choosing but we are not. Our choice is an inevitable one as a result of the reactions which have gone before. When we decide to do the right thing in a time of crisis, if the doctrine of determinism is correct, the only reason why we do so is because with our unique protoplasm, having its own particular heredity and environment, there was no other course open. No other decision was possible with the organism which we possess,—an organism with an environment and a heredity unlike that of any other individual in the world. Man is an organism like all others. Reason is simply the result of the play of external circumstances upon a physicochemical machine. There is *appearance* of free will without the substance because we are unaware of the causative chain of events which has predetermined the decision. Our actions are, hence, largely tropistic. We go towards food, warmth, and water in much the same way that the lower animals and plants do. The chief difference is in the mechanism through which the stimulus must act; its greater complexity necessitates a more circuitous and less visibly direct route. According to the conception of mechanism, the principle involved and the results obtained are the same throughout the organic world; alcohol increases the phototropic sensitivity of plants (Montemartini, 1926), and even though the inebriate does not seek the lamp-post because he is positively phototropic, his action *may* be the result of *thigmo*tropism! The emotions are likewise the result of physicochemical laws which, to the mechanist, are in the same category as the reactions which result in the nourishment and reproduction of the individual.

Modern Vitalism.—In these mechanistic mazes, hypotheses and fact have not always been clearly distinguished, with the result that many elementary students have not realized just how far the mechanists have been able to prove their doctrines. The vitalists have rebelled against what to them is an unwarranted assumption. They are quite willing to follow the results of experimental research and to accept the findings of the laboratory method, they say, but they find themselves unwilling to admit that because certain processes are physicochemical all must be.

Among the modern vitalists a few outstanding figures should be mentioned. Driesch has been especially impressed by the processes of differentiation in growth and the various stages in the formation of organs. Why should development assume this orderly and progressive series of changes? He saw in organisms a driving force which he called an *entelechy* that guided the growth of the individual. John Calvin placed the forces which guided man's destiny without him, but nevertheless put him in a position where little was left to his own choice. The mechanists have similarly deprived him of freedom by binding him down to the physics and chemistry of his past. Driesch forms a compromise between these two methods of determinism. Organisms are guided in their development by forces *extra*-physicochemical but at the same time residing *within* the organism.

Bergson has been impressed by the vast creative power of nature which bursts out into all manner of living things. In this creative power he sees an *élan vital* or living impulse which cannot be expressed in physicochemical terms. These ideas of Bergson and Driesch may appeal to us or not depending upon our temperaments. They are more likely to appeal to the emotional side of man than to his reason, and they find their best expression in the aspirations of the race as stated in poetry (which may possibly be nearer the truth than the products of reason).

In addition to these outstanding figures, there are many others who call themselves neo-vitalists. They do not attempt to separate the organic from the inorganic world on any definite set of characters, but they feel that life phenomena are unique because they deal with a unique material,—protoplasm. Just as the phenomena and characteristics of water are different from those of the hydrogen and oxygen that compose it, so protoplasm has unique properties because it is a unique substance. This conception, which is related to the doctrines known as *holism* and *emergent evolution*, seems to satisfy many people, but it really amounts to an admission that the problem is incapable of a solution. It is another way of saying that "Life is what it is, and it, therefore, cannot be otherwise"; but this sort of reasoning seems to be more satisfying to philosophers than to biologists.

One may take various inorganic compounds and reproduce with them certain features of organic activity one at a time; but the characteristic of protoplasm is that it can do all of these things,—grow, reproduce, respond to stimuli, etc.—at once! Protoplasm is a peculiar substance and until it has been made *in vitro*, its secrets have not been yielded up to the inquiring laboratory worker. Furthermore, knowing the physics and chemistry of an apparatus does not explain it. One may know all the laws of the gas engine,—the question of ignition, feed, carburetion, etc.—but is the engine then explained? Also it is quite possible for a system to *appear* mechanical and still not be. If an observer from another planet could see the New York subway system with its trains at regular intervals, it would all *look* to be purely mechanical and automatic. But the more *mind* there is behind it, the more automatic and mechanistic it looks!

Mechanism has made rapid strides and has done much. It has made analyses of the organism and has shown that an enormous number of previously mysterious processes can be easily explained on a physicochemical basis. It is a matter of easy demonstration that "Man as an animal contains enough fat for seven bars of soap, iron enough for an eight-penny nail, sugar enough to fill a shaker, lime enough to whitewash a chicken-coop, phosphorus enough to make 2,200 match tips, enough magnesium for a dose of salts, potassium enough to explode a toy cannon, and sulfur enough to rid a dog of fleas, the total cost of which is about ninety-eight cents," which is not enough to cause megalocephaly. In many of our large museums, one may see on display in a separate case these various elements which go to "The Making of a Man," with a jar containing about 125 pounds of water thrown in to make the stock complete. This exposition of the value of the human organism is interesting but not convincing. There remain too many unsolved problems of major importance for us to jump so quickly to mechanistic conclusions at this "stage of the game."

Unsolved Questions.—Among these problems may be mentioned the one of the origin of life. Life has either come from inorganic matter or it was coexistent with it in the universe. Both points of view have supporters. Since the chemical elements of the organic world are the common ones of the inorganic, it is reasonable to suppose that the living came in some way from the dead. If life originated upon this planet, this is undoubtedly what must have happened, inasmuch as at the time of the planet's formation the conditions were not suitable for life as we know it to-day. Arrhenius has suggested that the beginnings of life came to this world from outside. Experiments have shown that spores in a dried condition can resist very low temperatures, and while it is barely possible that, driven by the pressure of light, such spores could go from planet to planet, this is purely hypothetical in a field where only hypotheses can at the present time be given.

A second problem is that of the structure and composition of protoplasm. It is known what elements are necessary for protoplasm and something of their proportions but, to date, this unique material has not been synthesized in the laboratory. This must be done before the question will be answered to the satisfaction of all parties concerned.

In the higher organisms other problems equally difficult remain to be solved. What is thought? Is it a secretion from the brain, a physicochemical reaction, or what? Psychology has done much to show us how the brain operates, what regions control certain muscular reactions, where the thought centers are located, but concerning the real nature of thought little has as yet been offered. There are people who believe that thought is the only *real* substance. To them there are two kinds of reality: material and immaterial. "Thoughts are things" which are as real as sodium and chlorine. The physical scientist, dealing with the physical realm, has a tendency to neglect this other world and to reason that because it cannot be touched by his instruments, therefore it does not exist. Whether thought is the only real substance is certainly a disputable matter, but it must be admitted, in all fairness, that even in our work in the laboratory we are never conscious of the external world directly but only through our *thoughts* of this world. As pointed out by Berkeley, what we are always comparing is not sodium and calcium but our *ideas* of these two elements. This may sound like quibbling, but to one who is interested in the fundamental nature of the universe in which he finds himself, these are very important distinctions.

Connected with this question is that of the nature of the *ego* or of personality. Is this personality the result of particular physicochemical reactions or is it something else? Do only organisms have personality or is it an attribute of inanimate nature as well? Has there been an evolution of this personality from the lowest organisms to the highest, or is it something which has come in only with the more complex manifestations of matter? Before definite answers can be given to any or all of these questions, much work remains to be done. The nature of the problem presents almost insurmountable difficulties, only a few of which may be mentioned in this short book.

Difficulties in the Way of a Solution.—1. *The vital equilibrium problem.* The organism is in a state of perpetual change. The substance which makes up any cell, like that which composes a waterfall, is constantly changing while the form remains the same. It thus exhibits what is called a condition of *vital equilibrium.*

One can examine the water before it enters the fall and after it leaves but not while in motion, or the fall ceases to exist. In like manner, in the complex equilibria of the organism, the worker seldom or never has the opportunity to pry into the mechanism while in motion. He can examine the elements which go to make up the organism and he can examine them after they have been fixed and stained, but he is no longer examining *living* material. The quality which made it different is not there. Is the motion itself the life? Is life merely the constant interplay of molecule with molecule and death the absence of such motion? What makes this interplay of parts? When a clock stops it can be started again. Why cannot the organism be started when it stops?

2. *The physical tools problem.* The natural scientist can concern himself only with the world of material reality. His work lies within the realm of the senses, and the only forces for which his tools are of any value are physical forces, that can be weighed and measured. *If there were* forces operating in the world of immaterial reality, they could not be demonstrated until they were changed into physical forces. One should realize that the senses are limited in their scope; the eye is sensitive to rays of a definite wave length, and it was consequently many years before the shorter waves, which the eye cannot perceive, were detected by other means. If such extraphysical forces exist, they can be manifested only through the known physical ones, and the fact that they are not perceived as such does not prove their absence.

3. *The egocentric predicament.* The fact that the mind, which reasons, is never in contact with the facts about which it reasons is also much to be deplored. All our knowledge must reach the brain in the skull through the nerve channels. The brain is never in direct contact with the external world. All of its information is secondhand, and there are numerous instances which show how unreliable this information often is. This is a real predicament but one with which we must content ourselves.

Mechanism a Working Hypothesis.—These problems are called to our attention not to bolster up vitalism or to detract from mechanism but to present the problem in its true light. The mechanists have done much more than the vitalists in solving our problems. The mechanistic hypothesis has been the most fertile and, in fact, is the only one which has advanced our knowledge of the physical world about us. But mechanism is a *method* and should not be taken as an *explanation* of the universe. If one does not go into his laboratory with the belief that the problem he is studying can be solved along the lines of the mechanistic principles with which he is familiar, then he might as well remain outside; he will not advance science by any other method of attack. On the other hand, because he has found that *many* of the problems of life have responded to his methods, he is equally unscientific (because he is inaccurate) who jumps to the conclusion *before it has been proved* that *all* of the problems of life will yield to the same treatment.

It has been very interesting to see that the extreme mechanists, like Loeb and his followers on the one hand, and the mystic vitalists, such as Bose on the other, are both working to the same end. Both schools have infinite faith in the unity of things. The mechanists say: "The world is one. Rocks, plants, and animals are all the same. They are all subject to the laws of *physicochemical machines*." The mystics say: "The world is one. Rocks, plants, and animals all are the same. They are all subject to the laws of *life*." The difference lies in the unifying principle. The end results in these antipodal cases may be the same, but their connotations and their applications to the exciting business of living are very different.

It has been equally interesting to observe that the physicists and chemists, whose work is on a firmer quantitative basis than biology, are much less mechanistic in their conception of the universe than many of the "cocksure" biologists, whose work is only *roughly* quantitative.

Conclusion.—The conclusion from this discussion is that the mechanistic method of attack in physiological and general biological problems, based on the assumption that facts are explainable by known laws, is the only way of discovering new truths. The worker in this case must use the mechanistic method for its pragmatic value, even though it has its limitations. It is applicable only to facts in the physical world of material reality and cannot pretend to offer anything in the realm of immaterial reality. The possibility that such a world exists cannot be denied, and until the nonexistence of this realm has been proved, the present advances in science, which have merely scratched the surface of the unknown, do not permit of a categorical answer in support of either side of this most important question. Unfortunately (?) we do not yet know whether we are climbing the foothills on the way to the top of a known and discoverable peak, whose sure ascent is only a question of time; or whether we are trying to find the gold that lies at the foot of an ever receding rainbow.

QUESTIONS

1. Why are chemists, physicists, and astronomers more often vitalists than the biologists?
2. Why do the biologists become involved in religious controversies more frequently than the physicists and chemists?
3. What common ground have the mystics and mechanists? Where do they differ?
4. What place have philosophical discussions in science?
5. Can one develop his *reasoning power?*
6. What is pragmatism? Why may mechanism be said to have pragmatic value for a biological research worker?
7. What are the causality and uncertainty principles of modern physics? What is their bearing on the problems discussed in this chapter?
8. What are the relations between the doctrines of *neo-vitalism, holism,* and *emergent evolution?* Are they "mechanistic" or "vitalistic"?

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