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PLANT PHYSIOLOGY

BY

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EDITED BY

R. B. HARVEY AND A. E. MURNEEK

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PREFACE TO THE FIFTH RUSSIAN EDITION

The main objective in preparing this volume has been to present, in a brief and compact form, to the students of our universities and agricultural colleges and to our future technical agriculturists clear and exact information concerning the life and vital functions of green plants. Such information should help them to understand the significance of various methods that are being applied in agricultural practice to increase the yield and improve its quality.

The ultimate purpose of the study of plant physiology is to control the growth and development of the plant in order to satisfy the need of mankind for plant materials as well as to provide forage crops for livestock. Such control is possible only on the basis of a fundamental study of the life of the plant as a whole, as well as in its separate constituent processes. Analysis of all the vital processes occurring in the plant is, therefore, a very important part of physiology, and experimentation forms the basic method for revealing the laws controlling the vital processes. But analysis should not obscure the synthetic problems of plant physiology or the presentation of the whole life of the plant as a united organism; on the contrary, they should be intimately coordinated.

The course in plant physiology in agricultural institutions should differ from university courses both in its content and in the form in which it is presented to the student. In such a course, first place should be given to agricultural plants belonging almost exclusively to the group of higher green plants. Therefore, peculiarities of the physiology of saprophytes, parasites, bacteria, fungi, and algae are scarcely dealt with in this book. Many of these questions are discussed with sufficient detail in textbooks of microbiology and phytopathology. A course in plant physiology—or, as it is being termed of late, agrophysiology—should include questions of special physiology of separate groups of agricultural plants, as well as questions of varietal physiology. Very often, differences in physiological properties, apparently of no great significance, prove to be of value in determining the usefulness of one variety or another for certain economic and agricultural conditions.

Another feature distinguishing the course in agrophysiology is the detailed discussion of questions of ecology, or the interrelations between the plant and its surrounding medium. Without a study of these interrelations, it is impossible to understand thoroughly the life of agricultural plants or to conceive physiologically the different methods employed in cultivating plants. For most of these cultural methods are not directed upon the plant itself but affect it indirectly through altering environmental conditions, mainly the soil.

In the analysis of vital phenomena, plant physiology is based principally on physics and chemistry. That is why courses in these branches of science and especially in organic, physical, and colloidal chemistry must be studied before a course in plant physiology is undertaken. Sufficient information concerning the anatomy and morphology of plants is likewise essential. Without knowledge of the structure of the plant, it is impossible to comprehend the physiological processes taking place within it.

Plant physiology is an independent science based on the advance of other sciences, chiefly of physics and chemistry. It likewise serves in its turn as a basis for agricultural sciences. Success in agriculture depends on progress in physiology just as success in physiology depends on progress in physics and chemistry. I have endeavored to hold in view this significance of physiology for agriculture and have discussed with greater detail all questions that are of special importance in their practical application. At the same time, I did not find it suitable to emphasize questions that are discussed in courses in general agriculture, agricultural chemistry, agricultural microbiology, etc. For this reason, the subjects of assimilation of nitrogen by microorganisms, nitrification, fermentation, application of fertilizers, and the like are discussed very briefly in this volume, to avoid unnecessary and harmful repetition.

For the present fifth edition, I have thoroughly rewritten my original textbook. In the preceding editions, I followed the usual plan adopted in books on plant physiology of analyzing the life of the plant according to its separate functions: assimilation of carbon, nitrogen, and mineral elements; water relations; metabolism; respiration and fermentation; growth and reproduction. Such an arrangement of the contents, which almost excludes the possibility of considering the plant as a unit organ-

ism, undoubtedly represents one of the relics of mechanistic traditions in science. With every new edition, it dissatisfied me more and more until finally I decided to reorganize fundamentally the whole plan of the textbook.

In the present fifth edition, the discussion is arranged in such a manner as first of all to acquaint students with the general physicochemical foundations of the plant, with its chemical composition, and with its mechanisms of digestion and metabolism. After these introductory chapters, we pass to a discussion of some of the more important vital functions, such as respiration and growth, which are most clearly manifested already from the first growth stages—the germination of the seeds.

This is followed by an examination of the primary synthesis of organic substances, which forms the most characteristic peculiarity of the green plants, the process of absorption of mineral elements necessary for growth, and the questions closely related to these processes, *viz.*, the water relations and translocation of substances through the plant. This part acquaints the students with the most important physiological functions, which form the basis of the vegetative phase of plant life and of the period of increase in size and of the accumulation of organic substance.

In the following chapters, the plant is discussed as a unit organism in which all the parts and all the processes taking place in them are closely interrelated. These chapters are devoted to an examination of the resistance of plants to unfavorable external agents, to problems of interrelations of different parts of the plant, to questions of physiology of development and reproduction and to seasonal phenomena in the life of plants. The material contained in this final part of the course (Chaps. X to XIV) has found as yet comparatively little room in textbooks, and, indeed, available data in this direction are very meager as compared with those contained in the first nine chapters of this volume, which comprise the usual contents of textbooks on plant physiology. However, the great importance of these problems for agricultural production has led me to devote no less attention to these questions than to those of the nutrition and growth of plants.

In disposing the different parts of the course in such a sequence as to begin with the processes most pronounced during the

germination of the seeds, further to continue with the most characteristic leading features of the vegetative period of the plant's life, and finally to terminate with the processes connected with reproduction and maturation of seeds, I have attempted to reproduce for the student as clearly as possible a complete picture of the life of the plant, not as a sum of separate physiological functions, but as a unified developing process beginning with the germination of seeds and terminating with the maturing of seeds newly reproduced by the plant. The normal process of the development of plants determines likewise the usual cycle of agricultural operations from the preparation for sowing up to the harvest. This arrangement of the subject matter should therefore contribute to a closer coordination of the course in plant physiology with agricultural practice.

The new arrangement of the material, as well as the recent advance in different problems of physiology and other related sciences, required considerable rewriting of the text and a certain increase in volume, especially because the last two editions were issued almost without changes after the edition of 1929. For the present edition, most of the chapters have been rewritten, and the rest have been reexamined and supplemented. Thus the fifth edition represents as compared with the four preceding ones an entirely new book.

It is to be hoped that this new book will be accepted both by students and by teachers with the same cordiality as the preceding editions.

The first four Russian editions of my textbook were out of print within eight years. During this period, there were issued three editions of a Ukrainian translation, under the editorship of the Academician N. G. Cholodny, a translation into White Russian, and a translation into Georgian.

An English translation of the second edition of the textbook, issued in New York under the editorship of Professors R. B. Harvey and A. E. Murneek, has been widely adopted in American universities and colleges. All this proves that my book was not one too many among textbooks on plant physiology and that it met well the needs of higher institutions by offering a brief exposition of the fundamental facts and principles of our science.

SARATOV,
September, 1935.

N. A. MAXIMOV.

EDITORS' NOTE

The English translation of Dr. N. A. Maximov's "Textbook of Plant Physiology," edited from the first and second Russian editions, was widely accepted for class use. In the present fifth Russian edition, Dr. Maximov has completely rearranged the text, rewriting a major part of the discussion, and has introduced new materials from the data of recent research. This edition has been published under the title of "Plant Physiology" so that it will not be confused by librarians and students with the former edition, entitled "Textbook of Plant Physiology," by the same author and editors. The discussions, data, and arrangement are so different as to make this distinction necessary. This is a completely new book, bringing up to date the results of plant physiological research throughout the world. The editors have introduced data and viewpoints from recent American publications. They have also revised the discussion, particularly where conditions applying in Russia especially were described, and thus have made the presentation of more widespread application. This text is, therefore, not just a translation but a new book, based on the translation of Dr. Maximov's fifth Russian text. This has necessitated the introduction of the viewpoints of the editors on many subjects, thus amplifying the presentation of the original author.

New illustrations and data have been used in many cases. In general, illustrations from publications in English have been chosen. References for collateral reading have been introduced after each chapter. These have been selected deliberately from publications in English, wherever suitable discussion can be found, and a few references to the classical works of plant physiology in other languages have been cited. The translation into English was made by Dr. Irene Krassovsky.

R. B. HARVEY,
A. E. MURNEEK.

UNIVERSITY OF MINNESOTA,
UNIVERSITY OF MISSOURI,
August, 1938.

CONTENTS

PAGE

PREFACE TO THE FIFTH RUSSIAN EDITION vii

EDITORS' NOTE xi

INTRODUCTION xix

CHAPTER I

PHYSICOCHEMICAL ORGANIZATION OF THE PLANT

1. Basic Features of the Structure and Composition of the Plant Cell 1
2. General Concept of the Colloidal State and Fundamental Properties of Cell Colloids 3
3. Properties of Proteins and Other Hydrophilic Colloids. Structure of the Cell Wall and of the Protoplasm 7
4. The Cell as a Colloidal System. Osmosis and the Properties of Cell Membranes 11
5. Permeability and Plasmolysis. Osmotic Pressure of the Cell Sap and Methods of Its Determination 16
6. Water Penetration into the Cell. Suction Tension and Methods for Its Determination 20
7. Composition and Structure of the Plasma Membrane in Relation to Permeability 25
8. Conditions for the Accumulation of Substances in Cells 28

CHAPTER II

CHEMICAL COMPOSITION OF PLANTS AND THEIR BASIC METABOLISM

9. Chemical Composition of Protoplasm. Reserve and Structural Substances 34
10. Carbohydrates 37
11. Reserve Fats, Lipoids, and Phosphatides. Organic Acids 46
12. Reserve Proteins and Products of Their Hydrolytic Decomposition 50
13. Hydrolytic Decomposition of Reserve Carbohydrates and Fats by Enzymes during Germination of Seeds 55
14. Decomposition of Reserve Proteins during Germination. Proteolytic Enzymes 59
15. General Properties of Enzymes. Methods of Their Isolation and Purification 61
16. Influence of the Condition of the Medium upon the Activity of Enzymes. Specificity of Function of Enzymes and Their Classification 66
17. Synthetic Processes during Germination. The Utilization of Carbohydrates and Fats in Germinating Seeds 69

PAGE

18. Metabolism of Proteins during Seed Germination. Processes of Decomposition and Synthesis. The Significance of Ammonium Salts and Asparagine 72

CHAPTER III

RESPIRATION OF PLANTS

19. The Respiratory Process 82
20. Methods of Studying Respiration. Intensity of Respiration 84
21. Substances Consumed during Respiration. The Respiratory Quotient 89
22. The Dependence of the Respiratory Intensity on the Conditions of the Surrounding Medium 91
23. Anaerobic Respiration and Alcoholic Fermentation 96
24. Relation between Fermentation and Respiration. Theories of Respiration of Palladin and Warburg 100
25. Energy Relations of Respiration. Oxidation-reduction Potential. 105

CHAPTER IV

GROWTH OF PLANTS

26. Growth of Plants. Germination of Seeds 112
27. Growth of Separate Cells and the Processes Involved. Stages of Growth: Embryonic Development, Expansion, and Internal Differentiation 115
28. Methods of Measuring Growth. General Laws of Growth and Their Mathematical Formulation 118
29. Growth Hormones; Auxin, Its Action and Chemical Composition. 123
30. Influence of Temperature on Growth. Cardinal Points. Thermophilic Organisms 128
31. Influence of Light on Growth. Daily Periodicity of Growth. Formative Influence of Light 131
32. Influence on Growth of the Degree of Saturation of the Plant with Water 135
33. Chemical Stimulants for Growth. Toxic Action of Pure Neutral Salts. Balanced Solutions 139
34. Influence of Gravity upon the Position of Growing Organs. Geotropism 143
35. Influence of Light upon the Direction of the Growth of Organs. Phototropism 150
36. Chemo-, Hydro-, and Thermotropism 155

CHAPTER V

CARBON ASSIMILATION

37. General Concept of Photosynthesis and Its products 160
38. Methods of Detection and Study of Photosynthesis 164
39. Plastid Pigments. Chlorophyll, Its Chemical Composition, Structure, and Properties 167

PAGE
40. Chlorophyll Formation. Etiolation and Chlorosis 173
41. Absorption of Light Energy by Chlorophyll 175
42. Quantitative Relations between Absorbed and Stored Energy in the Plant. Decomposition of Carbon Dioxide by Energy Obtained from Oxidation. Chemosynthesis 178
43. Chemical Reactions of Photosynthesis. Theories of von Baeyer, Willstätter, and Warburg 184
44. Structure of the Leaf as an Organ of Photosynthesis. Penetration of Carbon Dioxide into the Leaf 188
45. Dependence of Photosynthesis on Light Intensity. Sun and Shade Plants. Culture of Plants in Artificial Light 193
46. Dependence of Photosynthesis on the Amount of Carbon Dioxide. The Carbon Dioxide Cycle in Nature 201
47. Dependence of Photosynthesis on Temperature. The Interaction of Factors and the Law of Limiting Factors. Diurnal Changes of Photosynthesis 203
48. Intensity of Photosynthesis and Yield of Dry Matter. Significance of the Relation between Photosynthesis and Respiration ... 210
49. General Course of the Accumulation of Organic Substance by the Plant during Its Vegetation 214

CHAPTER VI

Assimilation of Nitrogen by Plants

50. Absorption of Nitrogen from the Soil. Nitrates and Ammonium Salts as Sources of Nitrogen 219
51. The Products of Nitrogen Assimilation and Its Connection with Photosynthesis. Primary Synthesis of Protein Substances 224
52. Assimilation of Molecular Nitrogen of the Atmosphere by Soil Bacteria 228
53. Assimilation of Molecular Nitrogen by Symbiotic Bacteria and Leguminous Plants 231
54. The Nitrogen Cycle in Nature. Artificial Nitrogen Fertilizers ... 235

CHAPTER VII

Absorption of Mineral Elements by Plants

55. Mineral or Ash Constituents of the Plant, Their Composition and Origin. Indispensable and Supplementary Mineral Elements 239
56. Sulphur and Phosphorus, Their Metabolism in Plants and Their Cycles in Nature 245
57. General Role of Cations and Their Specific Activity 247
58. Absorption of Mineral Elements by Roots 250
59. Mineral Nutrition of Plants Grown in Soil 252
60. Methods of Determining the Nutritive Value of Soils 258
61. Use of Mineral Elements by Different Plants. The Accumulation of Mineral Elements by Plants at Different Stages of Growth. Relation of Plants to Calcium Salts in the Soil 264
62. Effect on Plants of the Hydrogen-ion Concentration of the Soil. 268
63. Physiological Basis of Fertilization 273

PAGE

CHAPTER VIII

WATER RELATIONS OF PLANTS

64. General Concept of the Water Relations of Plants. The Amount of Water Used by Plants 278
65. The Absorbing and Pumping Activity of the Root System. Root Pressure, Guttation, and Bleeding of Plants 282
66. Absorption of Water from the Soil. Water-holding Forces of the Soil. Available and Unavailable Water 287
67. Influence of Environmental Conditions on the Absorbing Activity of the Root System. Methods of Studying the Absorption of Water by Roots. Absorption of Water by Aerial Parts of the Plant 290
68. Transpiration, Its Role in the Life of the Plant. Methods of Studying Transpiration 294
69. The Physiological Aspect of Transpiration. Its dependence on Environmental Conditions and Its Daily Changes 301
70. Leaf Structure as a Factor in Transpiration. Stomatal and Cuticular Transpiration 303
71. Stomatal Apparatus of the Plant, Its Movements and Methods of Its Study. Nonstomatal Regulation of Transpiration 305
72. Water Balance of Plants. The Phenomenon of Wilting and Its Significance 311

CHAPTER IX

TRANSLOCATION OF SUBSTANCES IN THE PLANT

73. General Concept of the Ascent and Descent of Water in the Plant 320
74. Osmotic Movement of Water 322
75. Translocation of Water in the Vessels. Forces Causing Movement of Water; the Action of Transpiration 325
76. The Rise of Water in the Trunk of a Tree. Cohesion of Water in the Vessels 328
77. The Rate of Water Movement and the Resistance of Wood to the Ascent of Water. Intermediate Motive Forces in the Translocation of water 334
78. Translocation of Organic Substances in Plants and Their Storage. 337
79. The Mechanism of Translocation of Organic Substances through the Plant. The Theory of Münch 339

CHAPTER X

RESISTANCE OF PLANTS TO UNFAVORABLE ENVIRONMENTAL CONDITIONS

80. Effect of Low Temperatures upon the Plant. Freezing of Plant Tissues and Processes Accompanying It 350
81. Frost Resistance of Plants. Hardening to Low Temperatures. Methods for Determining Hardiness 352

PAGE

82. Other Harmful Effects during Wintering: Exhaustion, Drowning, Heaving of Soil, Winter Desiccation. Physiological Basis of Selection for Winter Resistance 358
83. Effect of high temperatures on Plants. Heat Resistance 361
84. Effect of Water Deficiency on the Plant. Soil and Atmospheric Drought 363
85. Peculiarities of the Water Balance in Dry Habitats. Xerophytes, Mesophytes, and Hydrophytes 365
86. Effect of Environment on the Structure of Transpiring Organs. Xeromorphism and Drought Resistance 369
87. Physiological Basis of Irrigation 374
88. Injury to Plants from Excessive Humidity. Factors in the Resistance of Bog Plants to Waterlogging. Lodging of Plants, Its Causes and Means of Control 376
89. Injury to Plants from Excess of Salts. Physiological Peculiarities of Halophytes. Alkali Resistance of Crop Plants 378
90. Resistance of Plants to Fungi. Character of the Interrelations between the Parasite and the Host. Immunity 382

CHAPTER XI

INTERRELATIONS BETWEEN DIFFERENT PARTS OF THE PLANT. VEGETATIVE PROPAGATION

91. The Plant as a Unit Organism. Correlation 395
92. Artificial Propagation of Plants by Cuttings and Layering. Regeneration and Polarity. Root-forming Hormones 398
93. Grafts and Chimeras 403

CHAPTER XII

PHYSIOLOGY OF THE DEVELOPMENT OF PLANTS

94. Normal Cycle of Development of Higher Plants. Annual and Perennial Plants. Interrelations between Growth and Development 409
95. Conditions for the Formation of Reproductive Organs. Hypothesis of Klebs. Significance of Changes in the Chemical Composition of Plants 412
96. Theory of Lysenko on the Stages in Development of Plants. Yarovisation, Its Theoretical Foundation and Practical Significance 416
97. Influence of Length of Day on the Development of Plants. Photoperiodism 422
98. Physiological Differences between Winter and Spring Types, Early and Late Plants. Basis of Selection for Earliness 428

CHAPTER XIII

PHYSIOLOGICAL PROCESSES DURING FLOWERING AND RIPENING OF FRUITS AND SEEDS

99. Physiological Processes during Blooming. Respiration of Flowers. Nyctinastic and Seismonastic Movements 432

PAGE

100. Physiological Processes Accompanying Pollination and Fertilization. Germination of Pollen and Growth of the Pollen Tube. Phenomena Accompanying Termination of Blossoming. Shedding of Immature Fruit 437
101. Ripening of Fruit and Seeds and the Biochemical Processes Accompanying It. Artificial Acceleration of Ripening 442
102. Influence of Environmental Conditions on the Course of Ripening. Shriveling of Grain and Its Causes. Dependence of the Chemical Composition of Fruit and Seeds on Geographic Factors 447

CHAPTER XIV

SEASONAL PHENOMENA IN THE LIFE OF PLANTS

103. The Dormant Period of Plants. Autumnal Maturation of Buds and Wood of Trees. Shedding of Leaves 453
104. Artificial Breaking of the Rest Period. Significance of Forcing for Agricultural and Floricultural Practice 456
105. Conditions of Seed Germination 459

INDEX 465

INTRODUCTION

Plant physiology is the science that deals with the vital phenomena of plants. Its purpose is to acquaint us with the life of the plant and the processes occurring within it so as to enable us to alter the course of these processes according to our wishes and thus to control and govern the life of the plant and to obtain from it the greatest possible amount of products necessary for mankind. The utilization of plants for human needs being the chief problem of plant industry, a knowledge of the physiology of plants naturally represents one of its essential foundations.

For a sufficiently exact and complete understanding of the life of the plant, we must first of all reveal its inherent limitations, study in detail the separate constituent processes, and ascertain the importance of each of them in the development of the plant as a living organism. Furthermore, it is essential to analyze these processes fundamentally and to study the physical and chemical phenomena on which they are based. Physiology, however, cannot be restricted to this purely analytical problem. Analysis must be followed by the solution of a problem of synthetic character, *viz.*, the reconstruction of the life of a plant as a whole. This synthetic work should not be confined to a study of the natural laws regulating plants in general and outside of the influences of the natural environmental conditions. Physiology must solve the more concrete problem of reproducing the life picture of definite plants in environmental conditions where they can successfully perform their vital functions. This synthetic viewpoint leads, therefore, to the creation of a special physiology, which from a practical point of view forms a very important supplement to general plant physiology.

Though a part of the botanical sciences, plant physiology is also closely linked with animal physiology. Life phenomena in their fundamental aspects are similar in all living things. Many of the essential organic functions, such as respiration, nutrition, and irritability, are exhibited both by plants and by animals.

Hence, numerous attempts have been made to create a science of general physiology that should include the study of vital activities manifested by all organisms. Such an approach to the investigation of living beings, however, would leave out of consideration the specific nature of plants. A deeper study of the peculiarities of the life of plants requires, therefore, an independent analysis of the problems that refer particularly to their physiology.

Though closely related to biological sciences of a descriptive character, plant physiology differs, however, in that it is based on physicochemical sciences. Thus in its analysis of vital phenomena, in separating the reactions of complex processes, plant physiology constantly resorts to physics and chemistry, and its progress is closely connected with the growth of the physicochemical sciences.

As has been stated previously, plant physiology forms one of the most important foundations of the agricultural sciences. Every important advance in plant physiology should lead, therefore, to new success in the field of plant industry. Conversely, problems of agricultural character stimulate the study of closely connected physiological problems, agriculturists frequently participating in this investigation. Plant physiology is certainly indebted in its advance to the work of agriculturists, especially in problems of nutrition. The names of such agricultural scientists as Boussingault, Hellriegel, and Prianishnikov rank among the most famous in the history of plant physiology.

Of all agricultural courses of study, agricultural chemistry is the one that stands closest to plant physiology, so close that it is impossible to draw a definite boundary between the two. The problem of plant nutrition is closely connected with the problem of fertilization; and it is quite natural therefore that technical agriculturists are often obliged to solve problems of the physiology of nutrition, while physiologists participate in the elaboration of the problems of the application of fertilizers.

Knowledge of plant physiology is also most essential in solving problems of agronomy. Most of the agrotechnical methods represent no more than an endeavor to create around the plant favorable conditions that will ensure the highest possible yield. Such are the various methods of tillage for the purpose of creating a desirable soil structure or the different methods for the accumu-

lation and storage of water necessary for the plants in the soils of arid regions. Reclamation of lands by drainage of excessively wet soil, as well as irrigation of very dry regions, requires the assistance of plant physiology, which must help to estimate the requirements and peculiarities of the plants for the sake of which expensive improvements are made.

Plant physiology is also closely connected with plant breeding. The selection and creation of new varieties have the object of increasing the yield and quality of plant products. For systematically planned plant breeding, it is necessary to be acquainted with the physiological properties of the varieties, their earliness, their winter and drought resistance, etc. Only careful physiological study of varieties can reveal these properties, and that is why every large institute of plant breeding in Russia possesses at present a physiological laboratory. The chief occupation of such laboratories is the physiological estimating of the more important newly selected varieties and comparison of them with those that have already been accepted as standards.

In studying microbiological processes in the soil and in investigations of agricultural products, agricultural microbiology really represents a special physiology of bacteria and fungi. In college courses of plant physiology, physiological processes related to microorganisms are frequently discussed with no less detail than those peculiar to the higher green plants. Such a detailed examination of microbiological phenomena, which, by the way, are often more easy to analyze than the processes taking place in the higher green plants, is justified by the fact that many of them throw light on the nature of some of the basic functions of higher plants. Thus the study of fermentation has aided understanding of the most important process of respiration. In this case, it is difficult to draw a definite boundary between physiology and microbiology.

Finally, the study of plant physiology leads to the development of new methods of influencing the plant under field conditions with the purpose of increasing the yield and the resistance to unfavorable conditions, accelerating the development of the plant, and improving the quality of its products. Among these methods, mention must be made of yarovisation (vernalization), elaborated by T. D. Lysenko. The method of forcing plants by etherization, warm baths, and different chemical agents was

originally discovered and elaborated by physiologists and is now widely used, not only for ornamental plants, but also in potato culture, as are also the methods of application of electric light and other forms of radiant energy in plant culture. With further progress in physiology and its increasing unity and coordination with the practice of agriculture and with the gradual adoption of the methods of scientific agriculture, we have the right to expect from physiologists the creation of further new and valuable cultural methods, which may raise agriculture to an unforeseen prosperity.

PLANT PHYSIOLOGY

CHAPTER I

PHYSICOCHEMICAL ORGANIZATION OF THE PLANT

1. Basic Features of the Structure of the Plant Cell. Protoplasm as the Carrier of Vital Phenomena. Proteins and Lipoids as Its Chief Constituents.—The life of plants is an extremely complicated chain of phenomena that are closely combined into a unit of action and very finely coordinated with each other. At death, this coordination is suddenly disturbed; the characteristic process of the continuous metabolism of substances forming the living organism becomes confused; processes of synthesis are replaced by processes of decomposition; internal physiological connection between the different parts is lost; and the plant desiccates or decays, becoming the prey of microorganisms.

Such a close coordination of the most complicated changes is possible only through intricate organization of the plant body. Externally, this organization is manifested in the different organs of the plant body; these in turn may be separated into various tissues, and the tissues into separate cells. The differentiation of plants into organs, tissues, and cells forms the subject of courses in morphology and anatomy. A sufficiently detailed acquaintance with the structure of the plant is essential for an understanding of its vital functions. There will be an opportunity in the future to return to such details of the plant structure as are especially closely connected with its functions. Whatever these details may be, they should not obscure the general plan of construction, *viz.*, that every part of the higher plant is composed of a multitude of different kinds of cells. These cells may vary in size and shape and may perform different functions.

Each cell consists of the same important parts: the cell wall; and its contents, the cytoplasmic and nuclear structures. The cell wall is a sort of external skeleton of the cell, and it preserves

the characteristic shape of each of the cells. Vital functions belong only to the contents of the cell, specifically to that part of it termed the "protoplast" and composed of the cytoplasm with its plastids, mitochondria, vacuoles, etc., and the nucleus with its chromosomes, etc. The cell sap comprising the major part of the volume of the cell and various cell inclusions (such as crystals and starch grains) often present in cells are not living parts. They are products of the vital activity of the protoplasm not actively undergoing metabolic change.

Certain properties of the protoplast are closely connected with its physicochemical organization and chemical composition. A more detailed acquaintance with the chemical composition of the protoplasm will be obtained in Chap. II, where the chemical transformations occurring in the cell will be discussed. At present, it is sufficient to indicate that the principal substances composing the protoplasm are the proteins, which represent the basic carriers of vital phenomena. But pure isolated proteins never reveal any symptoms of vital activity. When extracted from the organism and deprived of their peculiar structure, they prove to be lifeless organic compounds, similar to fats, carbohydrates, and other substances that are always present in cells of plants. In order to serve as a substratum for life processes, proteins must be closely combined with many other compounds, first of all with lipoids, which are substances closely related to fats. Only in the presence of lipoids and other substances, however slight in quantity in comparison to the proteins, is it possible to maintain the structure of protoplasm, so essential for its functions.

Proteins are extremely complicated organic substances. Elementary analysis always reveals in them at least five chemical elements: carbon, hydrogen, oxygen, nitrogen, sulphur, and sometimes phosphorus and several other elements. Of these elements, carbon constitutes about 55 to 56 per cent; nitrogen, 15 to 18 per cent; hydrogen, 6.5 to 7.5 per cent; sulphur, 0.3 to 2.5 per cent; and oxygen 20 to 25 per cent. The protein molecule is complicated in structure and enormous in size. It contains both NH_2 and COOH groups, thus possessing the properties of both acids and alkalies. Under the influence of dilute acids and other agents, proteins disintegrate into a number of amino acids, which therefore are assumed to be the chief constituents

of the protein molecule. The amino acids constituting the proteins and the type of their interconnections will be further described in the discussion of the metabolism of cells.

The indispensable companions of proteins in the living protoplasm, the lipoids, are of very complicated chemical composition and high molecular weight. Most of them contain phosphorus and are termed "phosphatides." Chemically they are closely allied to fats, which are esters of glycerin and fatty acids. Lipoids contain in addition phosphoric acid and nitrogen-containing organic bases. Like fats, lipoids do not form true solutions in water but are soluble in organic solvents such as esters and benzene.

Solutions of substances having large molecules always possess the properties of colloids. The study of the structural peculiarities of protoplasm is therefore closely connected with a study of the colloidal state.

2. General Concept of the Colloidal State and Its Significance for Vital Phenomena. Fundamental Properties of Colloids.—The colloidal state, in terms of physical chemistry, is one of very fine division of the substance as a phase separate from the medium in which the minutely divided particles are dispersed. These dispersed systems may be divided into three types: (1) coarsely dispersed systems with particles of the dispersed phase not less than 0.1μ (1 micron = 1 thousandth of a millimeter); (2) colloidal systems in which the particles of the dispersed phase range from 0.1μ to 0.001μ; and (3) molecularly dispersed systems, in which the division in the dispersed phase is so great that it is represented by separate molecules or ions that have lost all connection with one another. The coarsely dispersed systems are mechanical mixtures whose heterogeneity may be readily observed under the microscope. Such systems are usually unstable. Their dispersed phase is either precipitated or rises to the surface on standing. With sufficient subdivision of particles, however, a greater stability is obtained. Examples of such stable mixtures are fine suspensions of clay, which do not settle for many days, or common India ink, a suspension of minute carbon particles in water.

Similar to suspensions are the emulsions, or mixtures of two immiscible fluids, *e.g.*, water and oil. Ordinary milk is such an emulsion, in which particles of fat in water may be seen under

the microscope. Artificial emulsions are readily obtained by shaking vigorously two fluids that as a rule do not mix or by diluting with water an alcoholic solution of a substance that is insoluble in water.

In this manner, Perrin prepared the emulsion of mastic (a resin) on which he carried out his famous investigations on Brownian movements. In true solutions, dissociation attains its highest point, and the substance is dispersed in the mass of the solvent in the form of separate molecules or even ions. At such a degree of dispersion, the system becomes very stable, and the dissolved substances can be separated from the solvent only with the greatest difficulty and at the expense of a considerable amount of energy. Such a separation may be observed in the process of freezing or evaporating of solutions; the resistance to separation is expressed in the lowering of the freezing point and the increase of the boiling point.

Colloidal systems occupy an intermediate position between coarse mixtures and true solutions. The degree of dispersion in colloids is considerably greater than in coarse mixtures, and the dispersed particles are much smaller, usually not exceeding 0.1μ. Accordingly, they are more stable than coarse mixtures. The particles of Naegeli (the micelles, or micellae) are, however, considerably larger than molecules. They represent solid or liquid aggregates that, owing to their small dimensions, have in aggregate an immense surface, separating them from the dispersion medium. Consequently, in colloidal systems an important role is played by surface phenomena, especially by adsorption. There are substances that possess such large molecules that even their true molecular solutions display properties of colloidal systems. Proteins, lipoids, and several other organic compounds belong to this group of substances.

Representing dispersed systems with relatively large particles, solutions of colloidal substances called "colloidal solutions," or "sols," differ considerably from true solutions. A most conspicuous difference is their relation to finely porous plant or animal membranes, such as bladder or parchment paper. Colloidal sols cannot pass through membranes, as their large micelles are retained in the pores, while true solutions may pass readily. Upon this difference depends the well-known method of dialysis, by which colloids may be separated from crystalloids. A vessel

covered with a bladder membrane or parchment paper is filled with the liquid to be dialyzed and is then immersed in another vessel in which the water is continually renewed. The crystalloids diffuse through the bladder and are carried away by the water current. The colloidal solution remains in the dialyzer (Fig. 1).

Colloidal particles distributed in the dispersion medium usually combine with the molecules of the medium. This phenomenon is termed solvation or if the dispersion medium is water, which is the case with plant cells, hydration. A similar connection of the particles of a substance with the particles of the solvent is likewise assumed by many chemists for true molecularly, or ionically dispersed solutions. The capacity of hydration differs widely in different colloids. Hydrophilic colloids, the particles of which are firmly linked with a large number of water molecules, are distinguished from hydrophobic colloids, which are either incapable of hydration or whose hydration is not stable. The colloids forming the major part of living substance, such as proteins, show very pronounced hydrophilic properties.

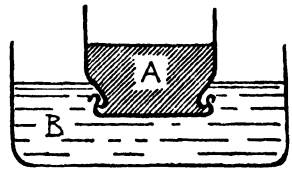


FIG. 1.—A dialyzer. Solution *A*, separated from water *B* by a semipermeable membrane.

Like the particles of coarsely dispersed substances, the colloidal particles dispersed in the dispersion medium usually carry an electric charge. The presence of such a charge may be proved by this very simple experiment. Immerse a pair of electrodes in the colloidal solution (sol), and pass an electric current through it. The colloidal particles will congregate around one of the electrodes, while the dispersion medium will accumulate at the other electrode. This phenomenon has been termed "electrophoresis." A quantitative study of electrophoresis shows that both phases of a colloidal system are equivalently but oppositely charged. In hydrosols, water is usually charged positively, while the colloidal particles carry a negative charge.

At the surface of contact of the colloidal particles with the dispersion medium, there arises a double electric layer. This layer is composed of the negative ions adsorbed by the particles and of the positive ions held by them in electrical equilibrium (Fig. 2). Naegeli used the term "micelles" for colloidal par-

ticles. At present this term designates the colloidal particle with all the ions retained by it. The magnitude of the electric charge obtained by the colloidal particle from the ions adsorbed on it is not constant but varies with the concentrations of various electrolytes in the dispersion medium, in the case of hydrosols, or sols in which water is the dispersion medium. If an electrolyte is added, the positive ions of which will be adsorbed on the colloidal particles more than the negative ions, an increase of the concentration of this electrolyte will cause a gradual decrease of the charge of the particles, until finally they will be entirely neutralized.

This will be at the isoelectric point of a given colloidal solution. The electric charge of the particles causes their reciprocal repulsion and determines to a considerable degree the stability of the colloidal solution. At the isoelectric point, when the charge is neutralized, the colloidal particles may be easily precipitated. With a further increase of the concentration of the electrolyte, the colloidal particles take on an opposite charge, and at the same time the stability of the colloidal solution increases.

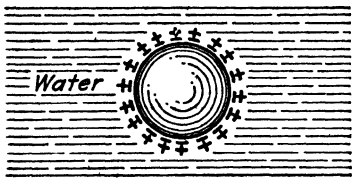


FIG. 2.—Electric charge on a colloidal particle in water, showing the double layer of + and − charges.

The dependence of the magnitude and kind of the electric charge of the colloidal particles upon the presence and concentration of electrolytes explains the great importance of the latter, in particular of hydrogen (and hydroxyl) ions, for the life of the plant. From the point of view of colloidal chemistry, protoplasm completely deprived of electrolytes could not exist, for the colloids forming it would be extremely unstable. The adsorbing force of colloidal particles for electrolytes is so high that the presence of even very small amounts of electrolytes insures a sufficient stability of colloids. Consequently, electrolytes form just as indispensable a part of the protoplasm as do organic colloid substances of the type of the proteins and lipoids. The general trend of the vital processes in the organism is to a considerable degree influenced by the complicated interrelations of colloids and electrolytes.

One of the most important changes that occurs in hydrosols under the influence of electrolytes is their coagulation. In

every hydrosol, there may be observed reciprocal repulsion of the colloidal particles carrying the same charge and likewise their reciprocal attraction, which is natural to all bodies. The magnitude of the reciprocal repulsion determines the degree of the stability of the colloidal system, while the reciprocal attraction, on the other hand, contributes to their aggregation into a complete mass of the micelles forming the dispersed phase. This mass is either precipitated or may rise to the surface. The separation of the dispersed phase from the dispersion medium is termed "coagulation" and may be caused by various factors.

One of these factors is the increase in concentration of electrolytes that neutralize the charge of the colloidal particles. As this neutralizing effect is caused by the adsorption of oppositely charged ions on the surface of the micelles, coagulation occurs only after the adsorption of a definite amount of ions, carrying a definite charge. The charge is determined by the valence of the ions; hence the general rule that the coagulating effect increases with the increase in valence. The same activity is shown by trivalent ions at lower concentrations than with bivalent ions and by bivalent ions at lower concentrations than with univalent ones (Hardy's rule).

When several electrolytes are added simultaneously to colloids, very often, instead of reciprocal increase of the effect, there may be observed an antagonism of ions. The addition of one kind of ions decreases the coagulating action of the others. Many investigators explain this antagonism by the fact that in a mixture of electrolytes colloidal particles adsorb both oppositely charged and identically charged ions, thus increasing their charge.

Another coagulating factor may be the increase in concentration of the colloid; with excessive decrease of the intermicellar spaces, the forces of reciprocal attraction may surpass the forces of electrical repulsion. One may mention also the senescence of the colloids, when the degree of dispersion gradually decreases.

3. Properties of Proteins and Other Hydrophilic Colloids. Gels and Jellies. Structure of the Cell Wall and of the Protoplasm.—Protein substances as well as several other closely allied hydrophilic colloids represent one of the most important group of colloids from the viewpoint of investigation of living substance, the protoplasm.

The conduct of colloids, when these are being acted upon by electrolytes and other agents, diverges appreciably from the regularities stated above. This is revealed chiefly in the study of hydrophobic colloids. It is obvious, therefore, that the main factor determining stabilization of hydrophobic colloids is the electric charge, while the main factor determining the stability of hydrophilic colloids is the capacity of the dispersed phase to adsorb various amounts of the solvent. Owing to the stabilizing action of the water layers surrounding each micelle, hydrophilic colloids may retain the properties of sols even at the isolectric point and precipitate (coagulate) only when acted upon by substances that extract water vigorously, *e.g.*, alcohol or acetone, and that are widely applied in biochemistry. This withdrawal of water explains the phenomenon of salting out of proteins and other hydrophilic colloids by concentrated solutions of neutral salts.

In most cases, coagulation of hydrophilic colloids is reversible. With the addition of water, the precipitate dissolves, forming a hydrosol. This distinguishes sharply hydrophilic from hydrophobic colloids; the coagulation of the latter is usually irreversible.

To understand the phenomena taking place in cells, it is of special interest to study the original coagulation of many hydrophilic colloids, which has been designated by the term of "gelatinization." Jellies have a very fine, complicated structure. Judging from some of their properties, it is to be assumed that the particles of the dispersed phase are continuous. Jellies are supposed to represent a fine network of colloid micelles, the interspaces of which are filled with molecules of the dispersion medium. This network structure accords well with the elasticity of jellies as well as with their property of swelling.

The process of swelling determines the capacity of jellies to absorb water or other corresponding solvents and thereby to increase considerably in volume. This phenomenon is especially marked in jellies previously dried, which may absorb the first portions of water with a force amounting to 1,000 atmospheres. This force may be measured by the weight that the swelling material is capable of lifting. Dry seeds are especially convenient for such experiments. In swelling, they develop such a pressure that they break glass containers. When placed in an

empty skull, they force it to separate along the sutures of the skull bones.

With increasing water content, the water-attracting force of the jelly gradually diminishes and finally becomes zero. This will be at the point of complete saturation with water. Some colloids retain the character of hard though gelatinized bodies even in a state of complete saturation and remain sharply distinct from the surrounding water. These are colloids with a limited swelling capacity, such as cellulose and starch. Protoplasm also belongs to the number of colloids that, though liquid, are limited in their swelling capacity. When in a state of complete saturation or even somewhat earlier, some jellies begin to show symptoms of dissociation of their micelles and finally dissolve completely in the solution. Such are, for instance, many protein substances, and gums (gum arabic). They are designated as colloids with unlimited swelling capacity. There is no sharply defined boundary between colloids with limited and unlimited swelling capacity. Thus, for instance, at a low temperature gelatin swells to a certain extent, while at increased temperatures it swells without limit. When heated, starch forms a jelly that changes into a colloid with unlimited swelling capacity.

The phenomena of swelling and dehydration play very important parts in the life of the cell. Most of the vital processes take place only in a protoplasm sufficiently swollen and practically saturated with water.

Both the cell wall and the protoplasm are complicated colloid systems, built up of ultramicroscopic elements invisible even under the most powerful microscope. The study of this structure requires therefore the application of ultramicroscopic and X-ray methods of investigation as well as the study of surface tension, viscosity, elasticity, and its other physicochemical properties. As yet all the details of the complex structure of these colloid systems are not fully known. Recent investigations, however, have revealed the more important basic features of their structure.

The cell wall, at least of the younger cells, consists mainly of cellulose, the chemical composition of which will be discussed somewhat later in Chap. II. At present it will only be indicated that cellulose is a polysaccharide of high molecular weight. It is built up of cyclic molecules of anhydroglucose ($C_6H_{10}O_5$)

that are connected by means of oxygen bridges. Each molecule of cellulose contains about 40 such cycles stretched in a chain, and as their number is not constant, it is natural that in application to cellulose and to similar polymolecular substances, the concept of the size of the molecule differs somewhat from its usual significance; thereby, the difference between molecules and micelles disappears. The length of such a molecule of cellulose approximates 200 angstrom units (Å.), and its molecular weight approaches 30,000 to 40,000.

The cellulose molecules arranged in chainlike form are combined in their turn into groups holding about 60 chains each and displaying a crystalline structure. The cell wall consists of such aggregates, arranged somewhat like bricks forming a wall.

In elongated fibrous cells, *e.g.*, in cotton or flax fibers, all the molecular groups are stretched in one direction, forming very fine fibrils. In cells of a parenchymatous character, the groups are formed with less regularity. Between these aggregates, there are interstices producing a finely porous structure of the cell wall.

The structure of the protoplasm is of a completely different character. Owing to the firm connection between the micelles, the cell wall has the character of a solid body and is capable of forming the particular shape of the cell, which is frequently of specialized type. Protoplasm itself is commonly a rather viscous fluid. When liberated from the cell wall, for instance, during the formation of zoospores in fungi and algae, it assumes a spherical shape. The streaming movements of protoplasm in cells that may be frequently observed demonstrate its liquid nature.

Although protoplasm is mainly fluid in nature, it is by no means a uniform liquid. It is an emulsion, the dispersed phase being composed of fats and lipoids, while the dispersion medium is a hydrosol containing a large amount of protein and other substances, as well as simple and complex ions. The substances forming the protoplasm are in a state of continuous interaction. Frequently they display partial coagulation and gelatinization, as well as the formation of a fibrillous structure somewhat similar to the structure of cellulose. The fibrils form a fine network interspersing the basic liquid mass of the protoplasm. The presence of such a fine network of ultramicroscopic fibrils gives

the protoplasm its viscosity and a certain degree of elasticity. It prevents its complete solution in water and limits its swelling capacity.

Depending on the course of the processes occurring in the protoplasm, its internal structure may change. With the partial solution, or with the condensation of the fibrils, there must be a corresponding alteration in the physical properties of the protoplasm, either a decrease or an increase of its viscosity, elasticity, etc. In the course of these processes, ionic reactions are of great importance, being accompanied either by a partial coagulation or a reverse solution of the colloids. Death of the protoplast is accompanied by a coagulation of its colloids and loss of its structure. In consequence, it loses the properties of a continuous, viscous liquid, and a watery phase separates from the coagulated hydrosol. At the same time, it ceases to retain the large amount of substances that were dissolved in the dispersion medium.

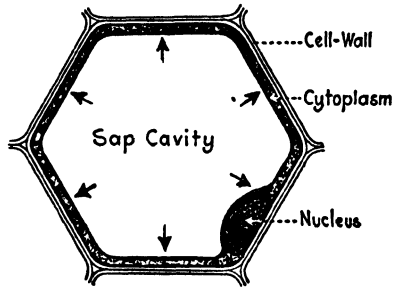


FIG. 3. —A diagram of an adult plant cell. Arrows show the direction of osmotic pressure (*after Sinnott*).

4. The Cell as a Colloidal System. Osmotic Properties of Cell Membranes. General Laws of Osmosis.—When very young, the whole cell is made up of a solid mass of protoplasm. In time, vacuoles containing a watery cell sap arise in this mass. These vacuoles finally merge, forming one central vacuole. The typical vegetative cell, therefore, may be considered as a double-walled sac filled with cell sap. The cellulose membrane of the cell is the outer wall of this sac, and the layer of semiliquid protoplasm forms a lining within it (Fig. 3).

These two membranes, separating the cell contents from the surrounding medium, are endowed with very different properties. The wall is a stiff porous gel. Owing to its solidity, it easily preserves its shape and has but a limited capacity for distention. Being a liquid, though viscous, the protoplasmic sac has an almost unlimited distention capacity. Not being able to maintain a definite shape, it readily fills all hollows and projections of the outer wall.

The properties of the walls delimiting the cell determine not only the form of it but also the laws governing the penetration of substances from the surrounding medium into the cell. These laws, in their turn, determine the conditions of absorption of nutrient substances by the cell. The presence of a rigid wall around every cell prevents the absorption of solid food. It is well known that one of the fundamental physiological differences between the animal and plant organism is that the animal cells (amoebae and infusoria) do not have a rigid wall and consequently are able to absorb and digest particles of solid food, while plant cells depend exclusively on substances dissolved in water.

Substances dissolved in water are frequently conceived of as being in a state approaching that of the gases. As has been shown, they are molecularly dispersed. Their separate molecules or ions are scattered among the molecules of the solvent. They have lost the power of cohesion and are in a condition of continual motion, similar to that of gas particles. Though this movement is considerably slower in the liquid than in the gaseous state, it leads to the same results. The particles of the dissolved substance tend to be uniformly distributed in the available space—the volume of the solvent. In other words, dissolved substances exhibit the phenomenon of diffusion. The slowness of the diffusion of such substances may be due to the rather high density of the medium in which they move, as well as to the fact that each of their molecules or ions is bound to the nearest water particles, which form around them a shell of water. Otherwise, the laws governing the diffusion of gases may be applied to diffusion of liquids in the cell. The fundamental law that the rate of diffusion is in inverse proportion to the size of the particles is likewise applicable here. The lower the molecular weight of a substance, the more rapidly will it spread through the solvent. The large particles of a substance of colloidal character move much slower than the molecules of crystalloids.

If a diffusing substance meets a membrane in its path, diffusion becomes more or less complicated. If this membrane is porous and the pores are filled with the same solvent, then there is less complication. In this case, however, the rate of diffusion is still more reduced. Only the largest colloidal aggregates are retained by the membrane. Membranes of this type are used in dialysis, by means of which colloids may be separated from

crystalloids. The cellulose of the cell wall is a membrane of this character. It is permeable to almost all substances dissolved in water.

If a sac made of a membrane, such as animal bladder, parchment paper, or a hardened film of collodion, is filled with a solution of a substance that does not diffuse very fast, *e.g.*, cane sugar, and if it is then tightly closed and placed in water, the sac will swell rapidly, its walls will become turgid, and finally it may rupture when it is unable to withstand the pressure from within. If instead of the opening being tied up, a glass tube is inserted in it, under the influence of internal pressure the level of the liquid in the tube soon begins to rise, first rapidly, then slower and slower, until it stops at a certain definite level, after which it begins to fall again.

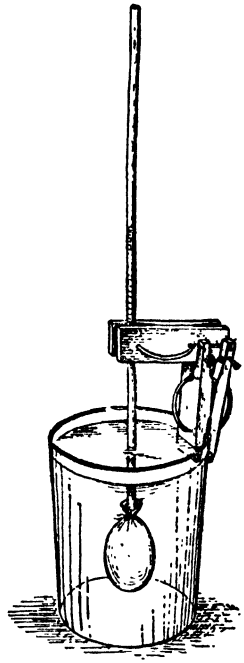


FIG. 4.—Osmometer (*after Dutrochet*).

By analyzing the changes in the contents of the sac, it is found that at first a rapid entrance of the water takes place, which leads to a considerable increase in volume. Afterward, the diffusion of the dissolved substance from the sac into the surrounding water becomes slower, and finally the concentration of the outer and inner solutions is almost equal, and the walls begin to collapse. The entrance of the water into the sac is called "endosmosis," the outward escape of the solute, "exosmosis." The whole apparatus, the sac with the inserted tube (Fig. 4), has been called an "osmometer" by Dutrochet, the French botanist, who was the first (1826) to study this phenomenon. The diffusion of liquid and dissolved substances through a membrane has been given the general name "osmosis." The hydrostatic pressure, developing as a result of an increase of endosmosis over exosmosis and swelling of the sac, is called "osmotic pressure."

Further investigations have shown that besides minutely porous membranes that only slightly check the diffusion of water and its solutes, there exist membranes that allow water or

the solvent to pass but that are wholly impermeable to the solutes. Such membranes, first discovered by Traube in 1867, are called "semipermeable" or "selectively permeable" ones. The semipermeable membrane that has been most studied is the precipitation membrane of colloidal copper ferrocyanide, arising at the place of contact of a copper sulphate solution with a potassium ferrocyanide solution. According to the opinion of many authors, the penetration of substances through such membranes takes place not by diffusion through the pores of the membrane but by solution of the substances in the membrane. The protoplasmic lining of the cell is a semipermeable membrane. The study of such membranes is of great importance in obtaining information about the processes that take place in the cell.

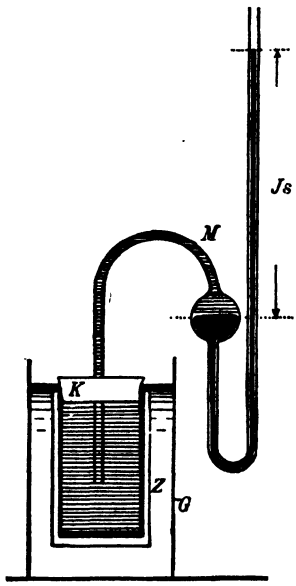


FIG. 5.—Pfeffer's osmometer. *G*, a vessel of water. *Z*, a porous cup with semipermeable membrane deposited in it. *M*, a mercury manometer. *Js*, height of mercury column indicates the osmotic pressure (*after Molisch*).

Such a study was first undertaken by Pfeffer in his classical "Osmotic Investigations" (1877). His work started a new epoch in investigation of the properties not only of semipermeable membranes but also of water solutions in general. Pfeffer showed that these membranes readily let through water but are quite impermeable to substances dissolved in it and that no exosmosis takes place through them. Therefore, the pressure developed by solutions contained within such membranes is not temporary but permanent and attains considerable magnitudes. A 2 per cent solution of cane sugar, for example, produces an osmotic pressure equal to 102 cm. of mercury, *i.e.*, above atmospheric pressure; while in Dutrochet's osmometer, even with very concentrated solutions, a pressure of 10 to 15 cm. is obtained with difficulty.

The osmotic pressures developed even by weak solutions are quite considerable. The precipitation membranes of copper ferrocyanide, on the other hand, are very delicate and easily

ruptured. Hence, in measuring these pressures, Pfeffer proceeded in the following way. He took a porous porcelain cylinder such as is used in batteries, filled it with a solution of copper sulphate, and submerged it in a solution of potassium ferrocyanide. A membrane was precipitated in the small pores of the porcelain, the walls of these pores providing a sufficiently strong support for the membranes to sustain a pressure of several atmospheres (Fig. 5).

Pfeffer's osmometer shows a certain similarity to the plant cell. In the latter also is found a readily permeable yet solid support in the form of the cell wall and the adjoining layer of semipermeable protoplasm. Therefore, the osmometer has frequently been called an artificial cell. However, in comparison with a real cell capable of being distended, Pfeffer's osmometer has rather rigid walls, and naturally it is unable to alter in volume.

In studying the influence of different factors upon the magnitude of osmotic pressure by means of his osmometer, Pfeffer established that it increases in direct proportion to the concentration of the solution and to the absolute temperature. Thus, it is governed by the laws of Boyle and Gay-Lussac, established for gaseous pressures.

In spite of the unity of the laws governing osmotic and gaseous pressures, these two pressures show certain differences, the chief of which is that gases always develop a pressure on the walls of the container enclosing them, while the osmotic pressure of solutions shows itself only in case they are placed in a container with a semipermeable membrane separating the solution from water. When placed in a glass or even in an osmometer not immersed in water, the most concentrated solution of any substance does not display any osmotic pressure on the walls. Osmotic pressure arises only when the solution and solvent are separated by a semipermeable membrane.

The osmotic pressure produced when an osmometer is immersed in water is explained by the attraction between the molecules of the solvent and those of the solute. Water enters the osmometer under the influence of this affinity and distends it, creating pressure on the walls.

If the water penetrating into the artificial cell is forced to lift a mercury column, as is the case in Pfeffer's osmometer, the rise ceases when the pressure of the mercury column balances the

tendency of the solution to increase its volume by the absorption of water. The height of the mercury column indicates the magnitude of osmotic pressure that may be determined as the equivalent of the external pressure to which the solution must be subjected to prevent the penetration of the solvent through the membrane, *i.e.*, to stop the increase in the volume of the solution occurring at the expense of the water particles penetrating into the osmometer. It is this external pressure that is usually measured in determining the osmotic pressure of a solution.

5. Impermeability of Plasma and the Phenomenon of Plasmolysis. Osmotic Pressure of the Cell Sap and Methods of Its Determination.—The easiest way of convincing oneself that protoplasm is but slightly permeable, or is impermeable, to the substances dissolved in water is by observing cells having colored cell sap, such as those of a red beet root or the leaves of red cabbage. While alive, these cells may be kept in water for many days without losing their pigment. But as soon as the structure of the protoplasmic membrane is destroyed, *e.g.*, by boiling or even by heating at 60 to 70°C. or by the addition of some poison, the colored sap diffuses into the surrounding water through the cell walls. More exact analytical methods have shown that other substances dissolved in water, *e.g.*, the salts and sugars, are not lost to any extent from the living cells. Otherwise the very existence of submerged plants would be impossible.

The phenomenon of impermeability of the protoplasm to substances dissolved in water may be detected in another way, also. If a cell, preferably having colored sap, is placed in a sufficiently strong solution of some harmless substance, such as sugar or potassium nitrate, the following results may be observed under the microscope. First, the cell decreases somewhat in volume, after which the protoplasm begins to pull away from the cell wall. If the solution is very strong, the protoplasm will shrivel up into a ball-like mass containing the highly concentrated cell sap (Fig. 6). The space between the cell wall and the protoplasmic sac will be filled with the external solution, which has penetrated through the wall. This phenomenon of shrinkage of the protoplasm is called "plasmolysis."

The different stages observed in watching the cell in water or in a solution may be explained in the following way. The protoplasm is readily permeable to water and impermeable to

substances dissolved in the cell sap. When the cell is surrounded by pure water, these substances attract the latter, causing the protoplasmic sac to increase its volume. As a consequence of this increase in size, the protoplasm distends and becomes appressed to the latter (Fig. 3). This condition is obtained when the elastic tube of an automobile tire transmits the pressure of the air pumped into it to the more solid outer casing. As a result of this pressure, the wall of the cell is in a rigid condition known as "turgidity," and the volume of the cell is slightly increased.

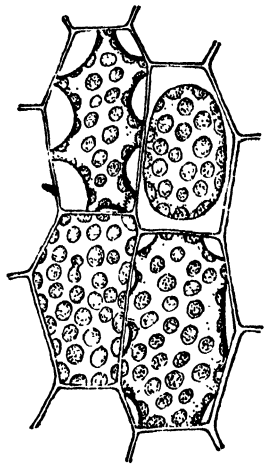


Fig. 6. Successive stages of plasmolysis in cells of a moss leaf (*after Molisch*).

If a cell is put into a concentrated solution of a substance, then owing to the impermeability of protoplasm, this substance will not penetrate into the cell sap but will remove water from it. The cell wall being permeable, the solution readily passes through it. Under the influence of the attraction, water escapes from the cell sap into the outer solution, and the plasma sac decreases in volume. If the total molecular concentration of the external solution is higher than the total molecular concentration of the cell sap, the pressure of the external solution directed inward exceeds the pressure of the cell sap directed outward. Consequently, turgor pressure disappears, and the cell decreases in volume. Moreover, the protoplasmic sac withdraws somewhat from the cell wall, which, with lessened internal pressure, contracts slightly.

The phenomenon of plasmolysis is of importance in many ways. In the first place, it shows a difference in the osmotic properties of the protoplasm and the cell wall. Second, it may frequently be used to indicate whether the cell under investigation is alive or not, because impermeability is peculiar only to living cells. Finally, plasmolysis enables one to determine with great accuracy the magnitude of the osmotic pressure within every living cell.

A method of determining osmotic pressure in cells was devised by De Vries as early as 1884. It is based on the following con-

siderations. The higher the osmotic pressure of the external solution above the osmotic pressure of the cell sap, the greater the shrinkage of the protoplasm. Conversely, the smaller the decrease in volume of the protoplasmatic sac, the less it will withdraw from the cell wall, thus showing a smaller difference between the two pressures. If a concentration of the outer solution is found that causes but an incipient shrinking of the protoplasm, which is usually observed in some corner of the cell, one then may assume that this concentration of the outer solution balances the concentration of the cell sap within. Since the concentration of the external solution is known, it is easy to calculate its osmotic pressure and from this the almost equal osmotic pressure of the cell sap.

The problem then is to find a concentration of the external solution that produces only incipient plasmolysis. For this purpose, a series of solutions of increasing concentration is prepared, differing from one another by the same magnitude, *e.g.*, by 0.1 gram molecule. Thin sections of the plant organ under investigation are placed in these solutions. The highest concentrations induce strong plasmolysis; the weakest, no plasmolysis whatever. Somewhere in the middle, a concentration will be found that will induce only the incipient stages of plasmolysis. The concentration of such a solution will only slightly surpass the osmotic pressure of the cell sap and may be assumed to be equal.

For computing the pressure in atmospheres, there may be utilized the equation mentioned above, according to which a solution containing 1 gram molecule of an undissociated substance per liter of solvent possesses an osmotic pressure of 22.4 atmospheres. But since this equation is applicable only to ideal very diluted solutions of such substances only as do not dissociate, it is preferable to use for various plasmolytic substances special tables that may be found in physicochemical reference books and that quote for each concentration the corresponding osmotic pressure, determined by precise experiment. It is customary to use cane sugar for determining osmotic pressure, for the determinations stated above are most accurate for this substance. Moreover it is quite harmless to the cell, and in conditions of plasmolytic experiments its penetration into the cell is excluded. In comparative experiments, such computations usually are not

made, indications being simply given for each object as to the isosmotic (*i.e.*, giving equal pressure) concentration of sugar in gram molecules. This magnitude, computed in atmospheres, is designated as "osmotic concentration."

This plasmolytic method of determining the concentration of the cell sap is simple and convenient and therefore widely used. However, it possesses many serious disadvantages. One of the main drawbacks is that the very procedure of plasmolysis, *i.e.*, immersion of the cells in concentrated solutions, causes a series of changes in them; in particular, it may produce a considerable increase of osmotic pressure. Furthermore, the plasmolyzing agent frequently penetrates into the cell, especially when solutions of nitrates or sodium chloride are applied. Another serious difficulty is the fact that the adjoining cells, even of the same tissue, frequently deviate in the magnitude of their osmotic pressure, and still more so the cells of different tissues, *e.g.*, the epidermis and the mesophyll of the leaves. This subject will be discussed in detail later on. For this reason, the plasmolytic method is being more and more often replaced by the cryoscopic method, *i.e.*, by determination of the freezing-point depression of the expressed sap. Since a gram-molecular solution of any nonelectrolyte decreases the freezing point of water 1.86°C. and since its osmotic pressure equals 22.4 atmospheres, there may be computed from the data of the freezing-point depression the total molecular and ionic concentration and the osmotic pressure in atmospheres. In the case of precise determinations, however, it is better to use special tables; for the actual relations in concentrated solutions, such as are represented by the plant sap, deviate rather widely from "ideal" magnitudes.

The absolute magnitude of osmotic pressure in the cells is quite high. In land plants, it most frequently ranges from 5 to 10 atmospheres; in submerged fresh-water plants, it is considerably lower, from 1 to 3 atmospheres. In plants native to saline soils or dry habitats, the osmotic pressure is very high, reaching 60 to 80, or even 100 or more, atmospheres. The magnitude of osmotic pressure depends chiefly on the accumulation in the cell sap of soluble substances of low molecular weight. In the majority of halophytes with an extraordinarily high osmotic pressure, the substances are most frequently either sodium

chloride or other mineral salts. In other plants, osmotic pressure is induced chiefly by sugars and organic acids.

Such immense magnitudes of the osmotic pressure in the cells appear improbable at first sight and give rise to questions as to how the thin cell walls are able to withstand the tension. A more detailed study of the question shows, however, that the magnitude of the osmotic pressure, calculated according to the concentration of the cell sap, considerably exceeds the actual hydrostatic pressure on the cell walls. The solution absorbed from saline soils, surrounding the cells of halophytes, shows a very high concentration, and the cell wall is subjected only to the excess of the inner over the outer pressure. If these plants are placed in pure water, the abrupt increase of hydrostatic pressure of the contents leads to a strong distention of the cell walls and sometimes to their rupture. This takes place but rarely, however, as with the distention of the cell its volume rapidly increases, and simultaneously the concentration of the cell sap and the osmotic pressure in the cell decreases. Osmotic pressure varies continually, depending on the chemical processes of the cell. Thus, when starch is hydrolyzed into sugar, the pressure is increased considerably. It is decreased as a result of the reverse process, the accumulation of starch at the expense of sugar. The imperfect oxidation of sugar, accompanied by an accumulation of organic acids of small molecular weight, *e.g.*, oxalic acid, also leads to an increase of osmotic pressure. By changing the chemistry of its internal processes, the plant is able thereby to regulate to a certain degree its osmotic pressure.

6. Laws Controlling the Penetration of Water into the Cell. Suction Tension of Cells and Methods of Its Determination.— The absorption of water by the cell from the surrounding medium is determined by the colloidal and osmotic properties of the cell. The phenomena taking place in dry seeds immersed in water or placed in a moist soil will first be examined.

First, the seeds will swell, considerably increasing in volume. Swelling is conditioned by the fact that both the cell walls and the protoplasm as well as the reserve food substances filling the cells of the seeds represent dry colloidal gels that attract water with great force. The limits of swelling are attained by various substances at different percentages of water content. The greatest swelling is displayed by protein substances; starch

swells less and cellulose still less. That is why the seeds of peas, soybeans, and other leguminous plants almost double their volume in the process of swelling. Seeds of wheat, rye, and other cereals rich in starch swell considerably less.

Completely dry seeds hold water with great force, exceeding sometimes 1,000 atmospheres. This suction force diminishes very rapidly with saturation. Thus, for instance, Shull observed in the seeds of *Xanthium* the following relation between the suction force and the water content in the seeds, in percentage of the dry weight: Air-dry seeds absorbed water with a force of about 1,000 atmospheres; seeds that contained 6 per cent of water, with a force of 400 atmospheres; seeds with 12 per cent, with a force of 130 atmospheres; seeds with about 30 per cent, with a force of 36 atmospheres; seeds with about 40 per cent, with a force of 15 atmospheres; and finally seeds containing about 50 per cent of water were saturated and did not show any suction force. The capacity of dry seeds to absorb water with great force and to extract it from adjoining more humid substrata is of essential importance for the process of germination. Seeds can extract water sufficiently for the beginning of their germination even from a comparatively dry soil.

Before germination seeds absorb water owing to the swelling of their colloids alone. This swelling usually leads to a bursting of the seed coat, which is composed of substances that swell less than the interior of the seed. But after the seed coat has burst, the rootlet and the other embryonic parts of the seed begin to grow rapidly, and vacuoles filled with cell sap appear in the interior of the cells. Further absorption of water by the germinating seeds is determined by the attraction of water by substances dissolved in the cell sap and which, as was stated above, determine its osmotic pressure.

If the cell walls were of unlimited extensibility, this absorption of water by the cell would continue until the concentrations of the inner and outer solutions became equal. But the cell wall, being limited in its extensibility and distending under the influence of the entering water, exerts on the cell contents an elastic counterpressure, opposite in its direction to the osmotic pressure and tending to counterbalance it. With further increase of volume, the pressure of the cell wall on the cell contents is augmented. Finally, the moment comes when the pressure of

the wall equals the osmotic pressure, and as a consequence a further increase in volume ceases. This condition of equilibrium may be called the state of complete saturation of the cell with water. If P denotes the osmotic pressure of the cell sap and T the pressure of the wall on the cell's contents, as a result of its strained condition, which is called "turgor pressure," it will be in the state of complete saturation with water, $P = T$, or, otherwise, $P - T = 0$. The water will neither enter the cell nor escape from it, no matter how great the sap concentration within the cell might be.

This condition of perfect saturation is natural to the cells of submerged plants, but it is hardly ever found in land plants. Because of the loss of water in the process of transpiration, their cells are never quite fully saturated with water and, hence, do not attain perfect turgidity. Part of their osmotic pressure remains unbalanced by the counterpressure of the cell wall; *i.e.*, P is always slightly greater than T. When such a cell is immersed in water, this additional osmotic pressure induces the entry of water into the cell and consequently leads to an increase in volume. This enlargement continues until the increased turgor pressure equals the magnitude of the osmotic pressure. For land plants, therefore, the formula reads as follows:

$$P = T, \text{ or } P = T + S,$$

or, finally,

$$P - T = S.$$

The value S is called "suction tension." It is this value, and not the absolute magnitude of osmotic pressure, that determines the absorption of water by the cell. As may be seen from the above formula, this value is the difference between the full osmotic pressure of the cell sap and the turgor pressure of the distended cell wall.

It can be found by experiment that the suction tension of each cell is not constant. It depends on the degree of saturation of a cell; for the more water it has lost in the process of transpiration, the higher will be its suction tension. Thus the plant cell possesses a self-regulating osmotic mechanism that absorbs water the more vigorously, the more it is in need of it. In the condition of complete wilting or of complete loss of turgor, the suction tension reaches its maximum and equals the magnitude

of the osmotic pressure of the cell sap, as may be seen from the formula $S = P - T$, where S varies inversely to T and reaches its highest value when $T = 0$.

The relationship between turgidity, osmotic pressure, and suction tension may be illustrated graphically by the following diagram (Fig. 7). On the abscissas are marked the various stages of tension of the cell wall at different degrees of saturation of the cell with water or at different volumes of the cell. On the left is seen the condition of a cell when completely plasmolyzed, or wilted, and, therefore, at its smallest volume, which is denoted by 1. The concentration of the cell sap, and conse-

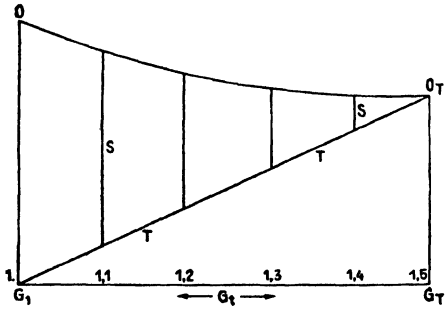


FIG. 7.—Diagram showing changes of the osmotic value in a cell passing from saturation with water to wilting (from right to left), and vice versa (from left to right): T, turgor pressure; O, osmotic pressure of the cell sap; S, suction tension; G, degree of distention of the membrane (*after Höfler*).

quently its osmotic pressure O attains its maximum. The cell wall is in a completely flaccid state, its pressure on the cell contents equals zero, and the suction tension S attains the magnitude of the total osmotic pressure. Now let the cell absorb water. Its volume, and consequently the tension of the cell wall, increases. This is denoted by the figures below the diagram. A gradually increasing turgidity T becomes noticeable. When the cell sap becomes diluted with water, its osmotic pressure falls, and the suction tension rapidly decreases. On the right side of the diagram is illustrated the condition of complete saturation with water; the suction tension having fallen to zero, the turgor pressure now balances the osmotic pressure of the cell. Under the average conditions of a cell, denoted in this diagram by the letter G, a part of the osmotic pressure is

balanced by turgor pressure. The other unbalanced part represents the magnitude of the suction tension of the cell.

This diagram clearly demonstrates that both magnitudes, the osmotic pressure of the cell sap and the suction tension, are not constant but change according to surrounding conditions and the internal state of the plant. However, osmotic pressure is considerably the more stable. While the suction tension falls with transition from wilting to complete saturation from its maximum to zero, the osmotic pressure decreases only 10 to 20 per cent. That is why, in characterizing the general properties of the cell, it is customary to use the average value of the osmotic pressure, but for stating the condition of the cell at some definite moment, especially in connection with rapid changes of the environmental conditions, a more precise picture is obtained from the data of suction-tension determinations. There will be occasion to return to this question in discussing the water relations of plants.

Methods of determining the suction tension of plant cells and tissues are mostly based on the same principle of the selection of equivalent concentrations of the external solution, which is applied in the plasmolytic method of determining the osmotic pressure. But in this case, a solution is found in which the volume of the immersed cell remains unaltered. Most of these methods have been devised by the Swiss plant physiologist Ursprung, who has contributed much during the last 20 years to clarification of the question of the suction tension of plants. The method most widely used for estimating the changes in the volume of cells is the measurement of the changes in the length of narrow elongated strips cut from plant organs such as leaves and immersed in solutions of neutral and harmless substances, mostly sucrose, at various concentrations. The osmotic pressure of the solution in which the length of the strip remains unaltered will be equal to the suction tension of the cells forming it. The suction tension is usually expressed in atmospheres of pressure. Changes in volume of separate cells are sometimes determined, but this can be accomplished only by difficult measurements under the microscope. Furthermore, the preparation of microscopic sections involves unavoidable injury to a part of the cells, and the sap escaping from them increases the values for the suction tension of the adjoining cells used for the measurements.

7. Permeability of the Plasma Membrane for Various Substances and the Question of Its Composition and Structure.—Thus far in discussing the osmotic properties of cells we started with the concept that the plasma membrane separating the cell sap from the surrounding solution represents an ideal semipermeable membrane, that it is easily permeable to water and absolutely impermeable to substances dissolved in water, whatever may be their properties. This schematic representation was essential for obtaining a clear picture of the general osmotic properties of the cell. Actually, however, details are considerably more complicated; in nature, there do not exist ideal semipermeable membranes, as there do not exist ideal gases, liquids, ideal solutions, etc. Each gas, liquid, or solution deviates more or less from ideal physicochemical laws; and every semipermeable membrane, being permeable to the solvent, is likewise somewhat permeable to the dissolved substances. Moreover, this permeability may differ for different kinds of substances. The plasma membrane likewise does not entirely prevent the diffusion of all dissolved substances; otherwise, the absorption of nutritive substances and their translocation from cell to cell would be impossible. The capacity of protoplasm to allow substances to penetrate through it is known as "permeability," and the study of this phenomenon is of the greatest importance for physiology. The simultaneous realization of permeability and impermeability of protoplasm is one of the most important contradictions in the life of the cell. A complete explanation of these contradictions will be possible only with a more consistent application of actual experimental results than has formerly been practiced.

De Vries, who first studied the phenomenon of plasmolysis, noted that some substances, *e.g.*, salts and sugars, produce a stable plasmolysis remaining for hours; while others, *e.g.*, glycerin or urea, produce a temporary plasmolysis. After several hours, the contracted protoplasm again distends and adheres to the cell walls. De Vries explained this phenomenon correctly. The substances slowly penetrate into the cell, their concentration outside and inside the cell becoming equal; and the distending influence of the substances contained in the cell sap again becomes manifest.

Further experiments have established that the number of substances that enter readily into the cell is rather large. Many

of them pass into the cell so easily and rapidly that no plasmolysis is induced. To this group belong chiefly the alcohols, the ethers, and some narcotics, such as chloroform. A careful investigation of various substances in respect to their capacity to penetrate into the cell drew Overton's attention to the fact that the protoplasm is readily permeable to those that are easily soluble in fats. Substances insoluble in fats penetrate with greater difficulty or not at all. And since the osmotic diffusion of a substance through a septum is determined, in the first place, by its solubility in the material forming the septum, Overton draws the conclusion that the protoplasmic membrane must be composed chiefly of fats or fatlike compounds, such as lecithin and other lipoids.

It must be noted that, according to the view held by Pfeffer and now shared by the majority of investigators, the properties of the protoplasmic sac are not uniformly the same throughout its extent. The external layer, the one nearest to the cell wall, and the internal layer lining the vacuole show semipermeability to a considerably higher degree than the intermediate layers. Pfeffer has called the external layers the "plasma membrane," or "Plasmahaut." In Overton's opinion, this surface layer consists chiefly of fatlike substances, which accumulate there because fats decrease surface tension. According to a fundamental law of physical chemistry, substances decreasing surface tension are bound to accumulate at the surface (Gibbs' law).

The lipoid theory of Overton, therefore, explains well the ready permeability of the cell to narcotics, alcohols, and esters; but it gives no answer to the question why water, though insoluble in fat, rapidly enters the cell, and why salts and sugars, which are readily soluble in water, penetrate but very slowly or not at all. Hence, this theory must be regarded as only a proximate idea.

Another theory of permeability, first proposed by Traube (1867) and again put forward by Ruhland, assumes that the structure of the protoplasm is similar to a very fine molecular sieve, or ultrafilter, letting pass small molecules but holding back molecules or molecular aggregates. This theory was developed by Ruhland chiefly on the basis of his experiments with the penetration of dyes, which did not show any parallelism between the solubility of dyes in lipoids and their permeability. Ruhland also assumed that undissociated molecules of salts

penetrate into the cells more easily than ions. He explains this by the fact that free ions are surrounded by a thick layer of water molecules, firmly held by electrical charges. His opinion, however, is contradicted by the fact that hydrogen and hydroxyl ions penetrate very rapidly and easily into the cell.

In general, the question of the permeability of protoplasm to different substances is very complicated and is far from its complete solution. A large number of substances essential for nutrition, *e.g.*, sugars and mineral salts, produce a permanent plasmolysis, which seems to show that these substances do not penetrate into the cell. This leads to the assumption that during plasmolysis the properties of the plasmatic membrane alter in the direction of a decrease of permeability. In this connection, the experiments of Sabinin are illuminating. He found that when the roots of seedlings are immersed in dilute solutions of different salts, all these salts are very soon present in the exudate that flows from the cut aerial parts of seedlings. Consequently, the root cells are considerably more permeable to salts than might be assumed on the basis of plasmolytic experiments.

These data as well as the ready permeability of protoplasm to water lead to the suggestion that the plasma membrane is built up not only of lipoids but also of protein substances that swell easily in water. Lepeschkin represents the plasmatic membrane as a rather unstable chemical combination of proteins and lipoids. Other authors suggest that these substances are not chemically combined but form a sort of complicated emulsion, as in the mosaic theory of permeability suggested by Nathansohn. It is of interest to note that the permeability of protoplasm to substances dissolved in water is not a constant magnitude but varies considerably according to external and internal conditions. Light, for instance, augments permeability of the plasma membrane, and concentrated solutions decrease it considerably. By means of certain stimuli, the permeability of protoplasm may be greatly increased. In the case of extremely sensitive plants, *e.g.*, *Mimosa*, these changes lead to sharp movements that are easily observed. This change of permeability perhaps is connected with the variations in the relative amounts of protein and lipoid particles in the surface layer. With the accumulation of the first, salt permeability increases; with the predominance of the latter, it decreases.

Thus selective permeability is the result of the very complicated structure of the living protoplasm, especially of its surface layer. This structure changes abruptly with the death of the cells and is followed by a sharp change in permeability. The surface layer of the protoplasm of killed cells instantly loses its capacity of retaining dissolved substances. Those dissolved in the cell sap of the dead cells easily diffuse out, while those dissolved in the surrounding medium penetrate with equal ease into the cell.

The loss of semipermeability by the killed cells involves alterations in the colloidal state of the protoplasm. There is a decrease in the degree of hydration. The micelles cling together in irregular aggregates, opening interspaces that are easily penetrable to water and solutions. This phenomenon as a whole is designated as "coagulation." Coagulation of protoplasm may be caused by different factors: the action of high temperatures; toxic substances; salts of heavy metals, acids, and alkalies; as well as dehydration and sometimes even simple mechanical pressure. In all cases of coagulation of protoplasm, death inevitably follows. This proves that the vital properties of protoplasm are closely connected with a high degree of dispersion of the colloids forming it.

8. Accumulation of Substances in Cells. Importance of Transformation of Substances for Their Accumulation. Conditions for the Accumulation of Substances in Cells without Alterations in Their Chemical Nature.—According to the laws of diffusion, such substances as salts and sugars, which are soluble in water and are absorbed by the cell, must continue to enter the cell until the concentration of each substance becomes equal, both outside and inside the cell. As the plant usually obtains very dilute solutions of nutritive substances, it is evident that their entrance into and accumulation within the cell depend upon conditions at present little known.

A most important prerequisite is the chemical change of the absorbed substances. For instance, when carbohydrates are stored in the tubers of the potato, the sugar obtained by the cells from the leaves is transformed directly into starch, which is insoluble in water. The concentration of sugar in the cells of the growing tuber is, therefore, extremely low and does not impede the diffusion of new amounts of sugar. The same is

the case in ripening oil-bearing seeds. The only difference is that in this instance fats are accumulated at the expense of soluble carbohydrates. Similarly, protein compounds are formed from amino acids. As a general rule, the substances entering the cell are subject to chemical transformation that assures their uninterrupted absorption. To demonstrate the dependence between the accumulation of substances in the cell and their transformation, one may use a sac of hardened collodion, representing a model of the plasma-membrane lining of the plant cell. Water diffuses readily through the collodion membrane, salts penetrate with greater difficulty, and colloidal substances hardly pass at all. If such a sac is filled with water and immersed in the solution of some salt, *e.g.*, in ferric chloride (Fig. 8), the salt will penetrate into the sac until the outer and inner concentrations become equal. No accumulation of iron within the sac will take place. If instead of pure water the sac is filled with a solution of tannin, which produces with iron a dark-colored compound of colloidal nature, the solution will remain inside the sac and will bind the iron ions that penetrate into the sac. As a result, the concentration of the iron ions inside the sac will be always considerably lower than on the outside, and finally the sac will absorb all the iron from the outer solution.

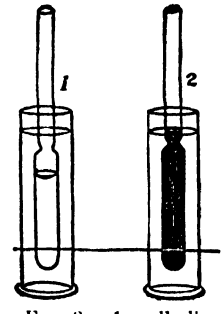


FIG. 8.—1, collodion sac with ferric chloride solution. 2, collodion sac containing tannin solution which reacts with iron ions to produce an inky solution.

As protoplasm is rather permeable to substances dissolved in water, these might be washed out from the cells. This leaching is prevented, however, owing to the fact that the reserve substances, starch and oil, are either insoluble in water or are in a colloidal state (protein and inulin). These substances are firmly retained by the cell. When seeds germinate or when organs containing food reserves are emptied, these substances are transformed into the former soluble and mobile condition. They leave the cells in which they had accumulated and move toward the growing organs.

This general mechanism of the absorption and accumulation of substances in the cell does not, however, always hold true. There are cases when soluble substances accumulate in great

quantity and remain in the same state in which they entered the cell. In the bulb of the common onion, for instance, considerable amounts of glucose are stored. In pigweed and other plants, much potassium nitrate accumulates, and its concentration in the root cells may exceed by far its concentration in the soil solution. Attempts have been made to explain this occurrence by the fact that sugar and potassium nitrate are present in these cells in special unstable combinations with some substances of the cell sap. All efforts at isolating such combinations have failed, however.

An example of this is the abundant accumulation of free inorganic ions in the cells of submerged water plants that are directly surrounded by water with a comparatively low salt concentration. By analyzing the cell sap of the fresh water alga *Nitella*, Hoagland observed the following amounts of the more important ions: chlorine, 106 milliequivalents per liter, sodium, 79; potassium, 58. The water of the pond where these algae grew contained: chlorine, 1 milliequivalent per liter; sodium, 1.2; potassium, 0.05. Thus the concentration of the ions of chlorine in the cell sap of the alga was 100 times, sodium 66 times, and potassium 1,160 times greater than in the surrounding water. Nevertheless, these ions remained in an entirely uncombined state. Analogous relations were observed by another investigator, Osterhout, in the cells of marine algae. As is well known, their cells accumulate large amounts of bromine and iodine, the content of which in sea water is quite insignificant.

Very often the explanation of these phenomena is sought in the membrane equilibrium explained by Donnan. It is not possible to give a detailed account of this equilibrium, which may be found in courses on physical and colloidal chemistry. Only the chief consideration on which Donnan's theory is based need be considered here. If a membrane impermeable to colloids and containing an easily hydrolyzable salt, one of whose ions is of a colloidal character such as sodium proteinate, is immersed in water, the sodium ions will not diffuse from the sac. They will be retained inside by the electrostatic attraction of the protein micelles. Now if some easily penetrating salt, *e.g.*, potassium chloride, is added to the water, then after some time equilibrium will be established between the four ions. At equilibrium, the ions charged oppositely to the colloid ion

(in this case, the cations) will be in a higher concentration inside than outside the membrane; while the ions similarly charged, the anions, will remain at a lower concentration inside than outside. According to Donnan's investigations, the product of the concentrations of the anions and cations of the freely diffusing salt must be equal on both sides of the membrane. In case there is a considerable excess of colloidal ions inside the membrane, there will be a nearly complete disappearance of cations (in this case, of potassium) from the surrounding medium. A corresponding amount of sodium will diffuse from the sac into the surrounding medium. On the other hand, anions (in this case, chlorine) will be almost entirely absent from solution inside of the membrane. This accumulation will take place in spite of the fact that both ions diffuse easily through the membrane and do not undergo any chemical reactions inside the membrane.

The protein substances, always present in cells, represent the colloidal ions mentioned above. Capable of carrying either a positive or negative charge, they can induce the accumulation of both cations and anions. And as the protoplasm is impermeable not only to colloids but also to many electrolytes, *e.g.*, to organic acids, these substances may cause the establishment of Donnan's membrane equilibrium between the cell and the surrounding medium and contribute to the accumulation of many cations in the plant in concentrations considerably exceeding those of the surrounding medium, *e.g.*, of the soil solution.

However, in connection with the continuous changes both quantitative and qualitative in the composition of the colloid substances, the conditions of Donnan's equilibrium are very complicated in cells and are far from being sufficiently understood. The attempts of several authors to reconstruct the whole problem of absorption and assimilation of substances in plant cells on the basis of the laws demonstrated by Donnan must be recognized as premature. During the last few years, there has been a gradual accumulation of data indicating that the process of penetration and accumulation of substances does not simply represent a purely physical process of penetration by osmosis but is rather an active physiological process, which requires the expenditure of a definite amount of energy by the organism. The recent works of Hoagland and his pupil Steward (1932), as well as those of Lundegårdh, Pirschle, Petri, and others, have

definitely established the closest connection between the penetration and accumulation of substances in the cells and the process of respiration, which represents the chief source of energy for all intercellular processes. Steward, for instance, observed that accumulation of bromine in the cells of potato tubers takes place only when a sufficient amount of air is bubbled through the solution in which the potato slices are placed. Just what the mechanism of the participation of respiration in absorption and accumulation of substances may be, is not clear. Some authors suppose that there is an adsorption of ions on the outer surface of the protoplast directly in contact with the surrounding solution. Owing to an internal change of the protoplasm, its particles move with the adsorbed ions to the inner surface and here transmit them to the cell sap. Others think that an important role is played by the carbon dioxide released in the process of respiration which leads to an increase of the hydrogen-ion concentration. These ions are then exchanged with cations present in the surrounding medium. According to this theory, developed chiefly by Lundegårdh, anions and cations penetrate independently of each other, using entirely different mechanisms and following different laws. These questions will be returned to when the subject of the absorption of mineral elements by the roots of plants is being examined.

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CHAPTER II

CHEMICAL COMPOSITION OF PLANTS AND THEIR BASIC METABOLISM

9. Chemical Composition of Protoplasm and of Other Parts of the Cell. Reserve and Structural Substances. The Main Groups of These Substances.—Protoplasm, the principal agent in vital phenomena, has long been the subject of chemical investigation, the purpose of which is to gain a more complete and detailed comprehension of its composition. Still, in spite of the efforts of many outstanding chemists, information concerning the chemical composition of protoplasm is very incomplete. This insufficient knowledge of one of the most important questions of biology is due to the extreme complexity and variability of the composition of the living matter. Life is a continuous exchange of substances and is impossible without these processes, and therefore the carrier of life, the protoplasm, must continuously change its composition, which greatly increases the difficulty of exact analysis. Besides, there are always found in the protoplasm various reserve substances that serve as a sort of fuel for the living factory and as raw material for the continuously created products. It is very often difficult to establish which of the substances revealed by analysis are substances constituting the protoplasmic essentials and which are only reserve or waste products. One of the essential difficulties in studying the composition of the protoplasm of plant cells is that it usually occupies a very small part of the total volume of the cell and is very difficult to separate from other substances present in the cell sap. The most convenient objects for the study of protoplasm are the plasmodia of slime molds (Myxomycetes), which are large aggregations of naked protoplasm. A careful analysis, performed comparatively recently by Kiesel (1925), gives the percentage composition of the plasmodium of one of these molds shown in the table on p. 35.

In this instance, of course, not only the active protoplasm but also a number of reserve substances have been included, as is

evidenced by the presence of glycogen and certain characteristic fractions of the fats and proteins. A full separation of the reserve substances from the active ones, however, is hardly possible, since for their work the active parts of protoplasm continually require reserve substances and, besides the reserves stored in special tissues, each cell contains a certain amount of them.

	Per Cent
Fats	17.85
Lecithin	4.67
Cholesterol	0.58
Mono- and disaccharides	8.06
Polysaccharides (largely glycogen)	17.02
Soluble nitrogenous substances	12.00
Proteins (including nucleoproteins)	20.65
Plastin	8.42
Nucleic acid	3.68
Fats from lecithoproteins	1 20
Unknown substances	5 87
Total	100 00

It is convenient to start the study of the chemical substances forming the plant and of their essential transformations inseparably connected with the vital activity of the plant by an examination of the chemical composition of seeds and of the processes of metabolism that develop during germination. One of the advantages of seeds for chemical investigations is the presence of comparatively large reserves of sufficiently uniform substances convenient for investigation. On the other hand, completely dry seeds represent dormant parts of the plant, in which all the chemical transformations may proceed at a very slow rate. That is why there is no danger during the process of isolation of the substances of their undergoing any marked changes by virtue of their vital activity. At the same time, in seeds that have begun to germinate the most important biological reactions proceed so intensely that they are most convenient for quantitative studies. Then too, in the beginning of germination, nearly all of the processes are restricted to those of decomposition, while later on they are accompanied by synthesis. This permits the study of these processes separately, a procedure very difficult if not impossible in investigating the life activity of adult parts of plants. Similarly convenient subjects for study are presented by other storage organs in plants, which serve

either for their supply in periods unfavorable for synthetic activity, *e.g.*, in winter, or for their vegetative propagation or for both purposes. Good examples of such structures are the fleshy roots, tubers, bulbs, and storage roots, or the storage tissues of tree trunks.

Although the reserve substances stored by plants in the various organs are quite varied in their chemical nature, three major groups, the carbohydrates, fats, and proteins, usually predominate. The first two are of a simple composition. Their molecules contain only three elements: carbon, hydrogen, and oxygen. They are, therefore, called the "nonnitrogenous compounds." The composition of the protein molecule, however, is more complex and contains, besides the three elements already mentioned, nitrogen, sulphur, and often phosphorus. In addition to carbohydrates, fats, and proteins, storage organs frequently contain various glucosides, phosphatides, alkaloids, organic acids, and many other substances. Their role, though very little understood, appears to be of a secondary character. Attention, therefore, will be centered here upon the examination of the transformation of the major groups, those interested in the minor substances being referred to more detailed texts of plant physiology or to special books on biochemistry.

In the seeds of various plants are found different quantitative proportions of the three major groups of reserve substances. Of the nonnitrogenous compounds, the fats and carbohydrates may replace each other in various plants. In the seeds of some, fats predominate; in others, carbohydrates, primarily starch. It is customary, therefore, to divide them into two groups, the oily and the starchy seeds. In the former, the fat content reaches 40 to 60 per cent, as the following table shows:

Oily seeds	Fat, per cent	Carbohydrates, per cent
Hemp	30 to 35	20
Flax	30 to 35	25
Sunflower	45 to 55	10
Poppy	40 to 50	25
Almond	40 to 50	20
Coconut	65	12
Castor bean	60 to 65	15

In starchy seeds, on the contrary, there is usually very little fat, as may be seen from these figures:

Starchy seeds	Fat, per cent	Carbohydrates, per cent
Wheat	1.8	69
Oats	5.3	60
Buckwheat	2.7	72
Pea	1.9	53
Corn	5.8	66
Rice	1.3	77

In his agricultural activity, man cultivates primarily plants with starchy seeds. He obtains these from cereals, which form the major part of his diet. In general, however, oily seeds predominate in nature, being produced by about 90 per cent of all plants. As they contain less oxygen and have a high energy value, about which details will be given later, oils form more concentrated reserves of nutritive substances than carbohydrates.

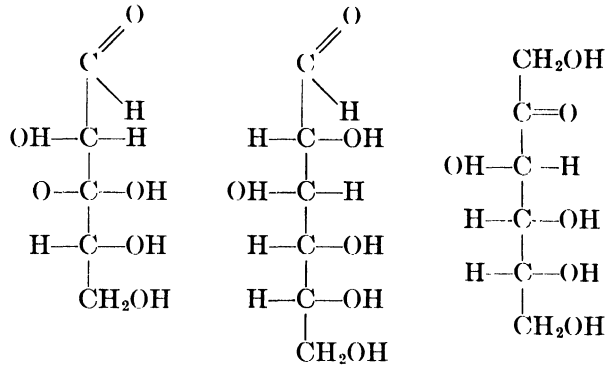
Protein compounds always form a smaller part of a seed's reserves, rarely reaching 25 per cent of its total weight. Oily seeds usually contain a larger percentage of proteins than starchy seeds. Hemp seeds contain 18 per cent of proteins; flax, 23 per cent; poppy, 19 per cent; almond, 22 per cent; and sunflower seeds, up to 30 per cent: whereas, in wheat, there is but 12 per cent; in buckwheat, 10 per cent; in corn, 10 per cent; and in rice, 7 per cent. The starchy seeds of leguminous plants, however, are rich in proteins, very much like the oil-containing seeds. Thus, peas contain 29 per cent protein; kidney beans, 23 per cent; lupines, 40 per cent; and soybeans as much as 44 per cent. This high protein content of leguminous seeds is undoubtedly connected with the ability of these plants to fix atmospheric nitrogen owing to the activity of the tubercle bacteria (see Art. 53). Having at their disposal during the course of their lives an unlimited supply of nitrogen, leguminous plants supply their progeny with a liberal amount of nitrogenous substances.

10. The Chief Representatives of Carbohydrates in Seeds and Other Parts of the Plant.—According to Kiesel's analysis, stated above, carbohydrates make up about 25 per cent of the total dry weight of protoplasm of slime molds. But in the body of

higher plants, the cells of which are covered with cellulose walls and nearly always contain reserves of starch and similar substances, carbohydrates usually form over one-half of the total plant substance. They represent a very large and extremely important group of organic products. The name of this group of substances is based on their being composed of three elements, carbon, hydrogen and oxygen, the two last elements being in the same proportion as in water; *i.e.*, for each atom of oxygen, there are 2 atoms of hydrogen. The empirical composition of carbohydrates may be expressed by the formula $C_nH_{2n}O_n$. In the primary sugars of the type of glucose, with the formula $C_6H_{12}O_6$, the number of atoms of oxygen is equal to that of carbon. In more complicated sugars, *e.g.*, in sucrose, $C_{12}H_{22}O_{11}$, it is 1 atom less; for these sugars are formed from the primary sugars by the removal of 1 molecule of water. With regard to their chemical properties, carbohydrates contain either aldehyde, —CHO, or ketone, $\begin{matrix} | \\ C{=}O \\ | \end{matrix}$, groups. They may be separated into two large divisions: primary carbohydrates or monosaccharides (monoses); and complex carbohydrates or polysaccharides (polyoses), representing a combination of several molecules of monosaccharides and formed by the removal of water. Depending upon the number of molecules of monosaccharides connected, there may be distinguished disaccharides, trisaccharides, etc. When the number of monose molecules forming the polyoses is not large, the latter retain some of the properties of the primary sugars, *viz.*, the capacity of crystallization and solubility in water; that is why they are often united into one group of sugars with the monoses. With an increase of the number of molecules, up to 6 or more, the polysaccharides obtain properties characteristic of colloids and do not give true solutions in water. Starch is one of the most important representatives of this group of colloidal polysaccharides.

According to the number of carbon atoms participating in the composition of the monoses, there may be distinguished trioses with 3 carbon atoms and the empirical formula $C_3H_6O_3$; tetroses, $C_4H_8O_4$; pentoses, $C_5H_{10}O_5$; hexoses, $C_6H_{12}O_6$; etc. Plants most frequently contain pentoses and hexoses. The former group contains xylose, arabinose, rhamnose, and others, while

in the latter group are found glucose, fructose, galactose, mannose, and others. Until recently, the structure of monoses was supposed to be represented by rows of carbon atoms with hydrogen atoms and hydroxyl groups attached to them. According to this assumption, the structure of arabinose, glucose, and fructose was represented by the following formulas:



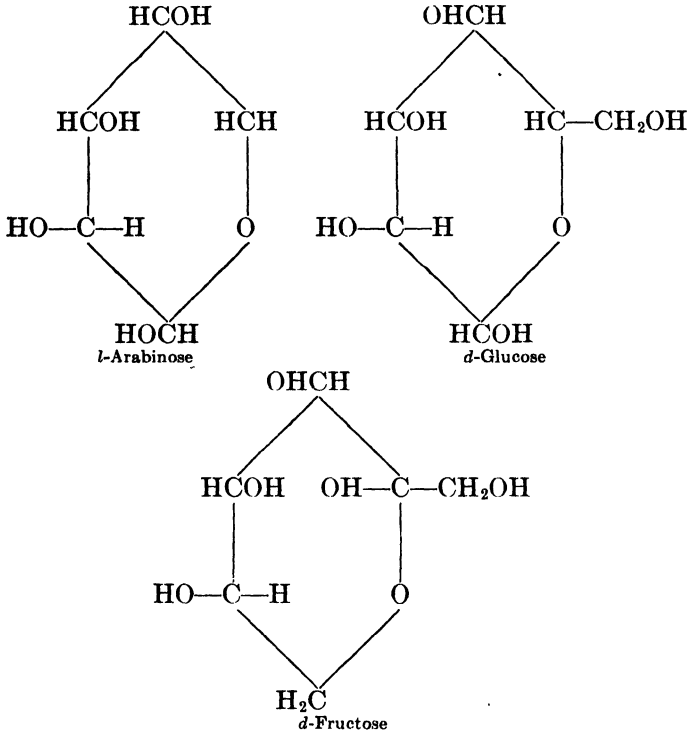
l-Arabinose *d*-Glucose *d*-Fructose

Recently, on the basis of their physical and chemical properties, which are too complex to describe here, it was suggested (Haworth) that pentoses and hexoses possess the structure of a six-membered ring consisting of 5 carbon atoms and 1 atom of oxygen (oxygen bridge). When only atoms of one kind are contained in a ring structure, the compound is said to be homocyclic; when different kinds of atoms are present, they are called heterocyclic compounds. This structure may be illustrated by the formulas shown on p. 40.

When the hydrogen and hydroxyl or other groups are arranged about the carbon atom in a symmetrical pattern, the compound is called symmetric. When they are arranged asymmetrically, the compound itself is asymmetric. Asymmetric arrangement of the atoms or atomic groups around the carbon atom gives the compound characteristic optical properties that can be used to identify it or to analyze its solutions.

Owing to the presence of asymmetric carbon atoms, all the natural monosaccharides are optically active and are capable of rotating the plane of polarization of light either to the right or to the left. On this capacity of rotating the plane of polarized light is based a simple quantitative method of determining sugars

in water solutions, by means of the polarimeter, which measures the angle through which the plane of polarization is rotated. This is widely applied in analyses in factories engaged in sugar production and other similar industries.



Glucose and fructose are very widespread in plants; there is not a single plant cell that does not contain some amount at least of one of these hexoses. As will be seen later, they represent the basic substance consumed by protoplasm in the process of respiration and participate in many other important biochemical processes.

Disaccharides are combinations of 2 molecules of monosaccharides joined through oxygen. Their formation is accompanied by the removal of 1 molecule of water, as illustrated by the following reaction:

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



(*From a portrait by Streblow*)

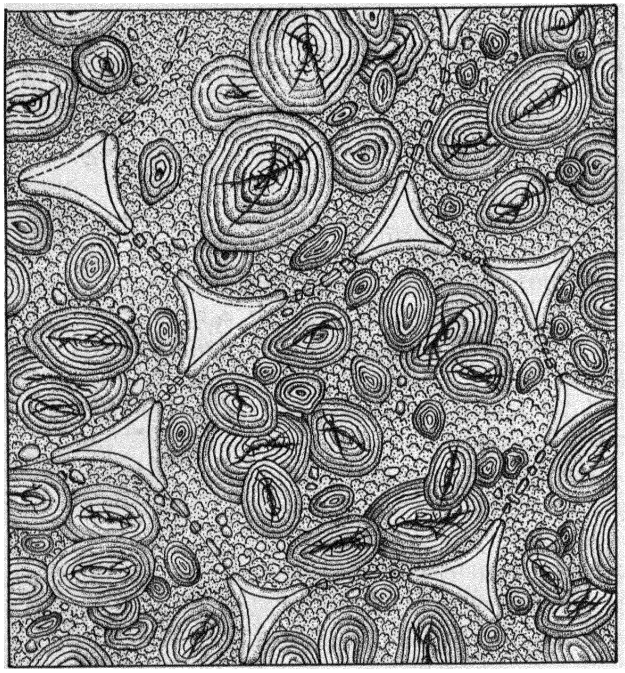
DR. NICOLAI A. MAXIMOV

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The most common dissacharide in plants is sucrose or cane sugar, which consists of 1 molecule of glucose joined to 1 of fructose. This sugar crystallizes readily and has an intensely sweet taste. It is frequently stored in the various organs of plants and is obtained in large quantities from the roots of sugar beets, which contain up to 20 to 25 per cent of sucrose, and, from sugar cane, sugar maple, and several other plants. By the action of dilute acid as well as of a special enzyme invertase, sucrose may be easily hydrolyzed; *i.e.*, by the introduction of 1 molecule of water, it disintegrates into equal proportions of *d*-glucose and *d*-fructose. This process is termed "inversion." From insufficiently precise methods of sugar analysis, the previous assumption was that plants frequently contained another disaccharide, maltose, which disintegrates on hydrolysis into 2 molecules of *d*-glucose. On the basis of further investigations (Davis, 1915–1916, and others), it has been established that maltose is very seldom found in the leaves of plants and in most cases is completely absent.

Closely allied to disaccharides is the group called "glucosides." These are compounds of sugars with other substances, such as alcohols, phenols, aldehydes, and acids. Glucosides are very widely distributed in the vegetable world. They are found very often in the food-storage organs. Hence, they may be regarded as special kinds of reserve substances, which at germination liberate the combined sugar. Amygdalin is one of the glucosides most prevalent in plants, being found in the seeds of the almond, peach, apricot, plum, apple, and others of the family *Rosaceae*. Hydrolysis of this glucoside liberates glucose, benzaldehyde, and hydrocyanic acid. The last splits off very readily and imparts to these seeds a bitter taste. The bark of various trees, as well as the seeds of lupine and several other plants, contains arbutin, which is composed of glucose and hydroquinone, and the roots of many plants contain saponins, poisonous glucosides, the nature of which is not yet fully understood. When combined with water, they give foaming solutions useful in industry, *e.g.*, soap root. The toxicity and sharp taste of many glucosides lead to the suggestion that they serve as protecting substances, preserving the seeds and other parts of the plant from being eaten or injured by animals.

There are α-glucosides, right rotating and split by the enzymes of yeast cells, and β-glucosides, left rotating and split by emulsin, which has no effect whatever on α-glucosides. The difference between these two types of glucosides is explained by the fact that the α-glucoside contains a more strongly rotating modification of glucose, the so-called α-glucose, while in β-glucosides the more feebly rotating β-glucose occurs.



D. KUBAN

FIG. 9.—Starch grains in cells of the embryo of pea.

Of reserve carbohydrates, the colloidal polysaccharides are the most widespread in plants. Their complex molecule is built up of a number of molecules of monoses. Because of the large molecules, the storage of polysaccharides has the advantage that its accumulation is not accompanied by an excessive increase of osmotic pressure in cells, which would rise still higher with the gradual drying of seeds. In this respect, disaccharides are more advantageous than monosaccharides. Solutions of the same percentage (on the basis of weight) contain only half the number

of molecules per unit volume, and consequently their osmotic pressure is only half that of the corresponding monosaccharides. This is probably the reason why monosaccharides, as compared with di- and polysaccharides, serve rarely as storage reserves.

Of all the polysaccharides, the most widespread in plants is starch. It is insoluble in water. Its empirical formula is $C_6H_{10}O_5$. It may be found in various tissues. Its presence in leaves is only temporary during the day and is the result of intense

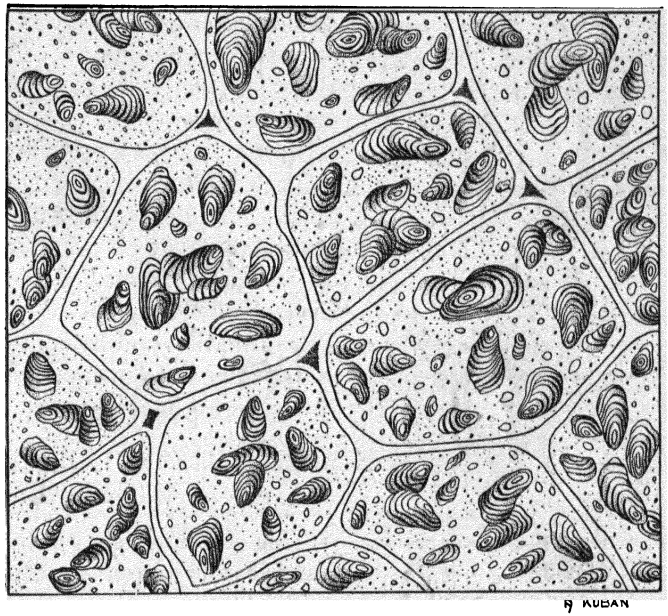


FIG. 10.—Starch grains in the cells of a potato tuber.

assimilation and consequent accumulation of carbohydrates that have not had the time to be translocated. Starch is likewise found in permanent storage tissues, *e.g.*, in seeds (Fig. 9), wood, and underground storage organs (Fig. 10), which are sometimes closely packed with starch. Being insoluble in water, starch accumulates in cells in the form of grains of various shape and size, characteristic of certain kinds of plants. Examination of the structure of the starch grains under the microscope will determine to what kind of plant they belong. This serves as one of the essential methods of determining the identity of flour

and distinguishing various admixtures (Fig. 11). In their internal structure, starch grains represent spherocrystals composed of very fine crystalline needles (trichites) closely packed and radiating from the hilum in concentric layers.

The formula $C_6H_{10}O_5$ does not express precisely the composition of starch. All the methods of determining the molecular weight prove with certainty that its particle is considerably larger and that its formula should be written $(C_6H_{10}O_5)n$. The cryoscopic method indicates an especially large starch molecule, and different authors give values to the magnitude n of 30, 108, 149, or 638, for various preparations.



FIG. 11.—Starch grains. Top, potato; center, corn and bean; bottom, rice and wheat.

Starch is hydrolyzed by the action of dilute acids and yields glucose; so its particle may be represented as a certain number of glucose molecules joined through the removal of water. The formation and hydrolysis of starch may thus be expressed by the following reaction:

$$n C_6H_{12}O_6 \leftrightarrows (n-1)H_2O + (C_6H_{10}O_5)nH_2O.$$

This formula shows that a particle of starch contains 1 molecule of H_2O more than is expressed by the ordinary formula $(C_6H_{10}O_5)n$.

If starch is hydrolyzed chemically, *e.g.*, by heating it with HCl, the formation of glucose proceeds in several steps; first appear the polysaccharides, soluble in water, which are termed dextrines; these are followed by the disaccharide maltose, which further disintegrates into 2 molecules of glucose. In living tissues of plants, however, hydrolysis of starch leads to an accumulation of sucrose, which involves the transformation of half of the glucose to fructose. The mechanism of this reaction taking place in living cells is as yet not clear, but it proves how much more complicated vital changes are than the chemical reactions that occur in the test tube.

Starch is not soluble in cold water, but it swells considerably by absorbing as much as 40 per cent of water. This water is retained with high imbibitional force. Even in an air-dry condition, starch will hold as much as 15 per cent of water. Starch has a high specific gravity (1.5 to 1.6). It precipitates easily and hence may be separated by settling, which is used in its purification. The starch grain seems to have a complex structure. The principal constituent of the grain, called "granulose," becomes more soluble upon heating in water and is easily hydrolyzed. The rest of the grain is less easily hydrolyzed. It remains in the form of a fine skeleton when the granulose fraction is dissolved. Both give the iodine reaction typical for starch, granulose, however, showing more of a blue, the skeletal part a violet shade.

Cellulose is a carbohydrate closely related to starch. It forms the basic substance of cell walls and has the empirical formula $C_6H_{10}O_5$. Cellulose differs from starch in being considerably more stable chemically. It is not soluble in hot water and can be hydrolyzed with difficulty by dilute acids. The end product of the hydrolysis of cellulose, as in the case of starch, is glucose. The intermediate product is likewise a disaccharide but, instead of maltose, it is cellobiose.

The arrangement of the molecules of cellobiose in the cellulose, like that of maltose molecules in starch, is chainlike, consisting of rows of constituent units arranged in a definite space lattice.

In elongated plant cells, *e.g.*, in bast fibers, all these chains are situated parallel to each other and at the same time somewhat inclined in relation to the longitudinal axis of the cell. In consequence, the fibrils are disposed in spirals, which may be shown by means of an ultramicroscope or X rays. Sometimes the so-called "streakiness" is perceptible under an ordinary microscope.

Owing to its chemical stability, cellulose forms the basic skeleton of the cell wall and does not represent a reserve carbohydrate. The role of a storage substance belongs to certain secondary deposits on the wall designated "reserve cellulose" or "hemicellulose." This is stored in the endosperm and cotyledons of many seeds in the form of substantial thickenings of the cell wall, which lend to the entire tissue considerable

stability. Hemicellulose is found in the horny endosperm of certain palm seeds (*Phytelephas* and *Coelococcus*), the so-called "plant ivory" of which buttons are made. A similar structure is displayed by seeds of the date palm, coffee, peony, lupine, nasturtium, and several other plants. The chemical composition of the reserve cellulose varies in different plants. It contains various pentosans, anhydrides of polysaccharides composed of pentoses, most frequently of xylose and arabinose. These are

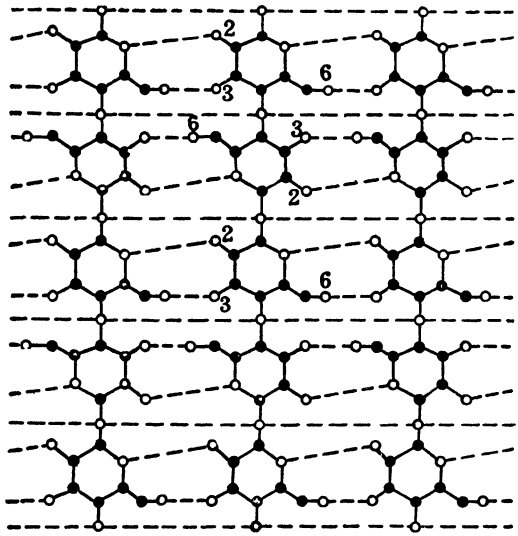


FIG. 12.—Tangential section through a ramie fiber showing three chains of glucose units. Dark lines indicate primary valence bonds; broken lines indicate probable general direction of secondary valence forces (*after Sponsler*).

designated correspondingly as xylans and arabans. Of the hexosans, it contains mannan and galactan, *i.e.*, polysaccharides which on hydrolysis disintegrate into the hexoses, mannose and galactose. Hydrolysis of reserve cellulose proceeds very readily by enzyme action. In germinating seeds rich in hemicellulose, it is dissolved very rapidly.

11. Reserve Fats, Lipoids, and Phosphatides. Organic Acids.—Fats and oils are found in almost all seeds. They enter into the composition of the protoplasm of embryonic cells. In most seeds, they are especially abundant in the embryo, representing reserves readily available during germination.

Fats are complex esters of glycerin and fatty acids either of the saturated or unsaturated series having the general formulas $C_nH_{2n}O_2$, $C_nH_{2n-2}O_2$, $C_nH_{2n-4}O_2$. Saturated acids, such as stearic and palmitic, give solid fats, while unsaturated acids, such as oleic, linoleic, and linolenic, form mostly liquid fats. Natural oils, found in seeds, are composed mostly of a mixture of different fats. In this connection, it is of interest to note that in plants of warm climates fats with a higher melting point predominate, *e.g.*, the oil of cacao, coconut, and other tropical products; but in plants of a temperate zone, such as hemp, flax, poppy, and others, liquid unsaturated fats predominate. Unsaturated fats are easily oxidized in the air, usually forming a hard film on the surface; hence, they are called "drying oils" and are used in the manufacture of oil paints.

Through the action of alkalies and acids, fats are easily hydrolyzed, even at low temperatures, through the addition of 3 molecules of water and breaking up into glycerin and a fatty acid.

The hydrolysis of palmitin, the fat of palm oil, for instance, proceeds according to the following formula:

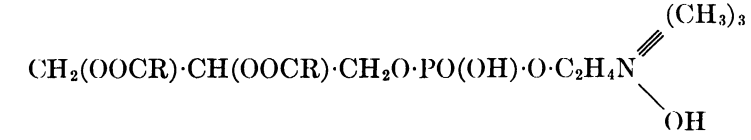
$$\underset{\text{Palmitin}}{C_3H_5(C_{16}H_{31}O_2)_3} + 3H_2O \rightarrow \underset{\text{Glycerin}}{C_3H_5(OH)_3} + 3\underset{\text{Palmitic acid}}{C_{16}H_{32}O_2}.$$

This process is known as "saponification." It is applied in industry in the manufacture of soap (sodium palmitate, etc.) and glycerin.

As reserve substances, fats have a number of important advantages over carbohydrates. Being insoluble in water, they readily become inert reserve substances, thus entirely disappearing from the sphere of other reactions and processes going on in the cell. Being liquids, they can completely fill the tissues of reserve organs. Moreover, they do not contain hygroscopic water, of which 15 to 20 per cent is present in reserve carbohydrates as a useless weight in dry seeds. Finally, they contain very little oxygen and consequently upon oxidation develop a large amount of heat per unit weight. When 1 g. of fat is oxidized in the organism, or outside of it, there is obtained on the average 9.3 cal. of heat, while 1 g. of protein will give 5.7 cal. and 1 g. of starch only 4.1 cal. These properties make oils extremely valuable reserve substances, and hence it is but

natural that in 90 per cent of all plants the seeds belong to the oily type. The only advantage possessed by reserve carbohydrates lies in the fact that without any complex chemical changes they can be converted into glucose and monosaccharides in general, substances of ready availability and great usefulness to plants, while fats must undergo many transformations before they can be utilized.

A group of substances closely related to the fats are the lipoids, or fatlike substances. The most important representative of this group is lecithin, a complex ester composed of glycerin, two fatty acids, and phosphoric acid, which in its turn is united with a strong, nitrogen-containing, organic base, choline. The general formula of lecithin is:



in which RCOO represent the various fatty acid radicals.

Lecithins are found in all living cells, animal as well as plant. They are not reserve substances but enter into the composition of the protoplasm as a necessary integral part of it. There is reason to believe that they accumulate especially in the so-called "plasma membrane." Overton, as has already been noted in Art. 7, attributes an outstanding function to them in the determination of the osmotic properties of this membrane. Some authors, Palladin, for instance, attribute to lecithins and to other closely related substances, the so-called "phosphatides," an important role likewise in the oxidation processes during respiration. Finally, having the capacity to form easily complex compounds with a great variety of substances, particularly the proteins, lipoids may have an important role in the synthetic processes of the cell. All these, however, are only suppositions of a hypothetical nature. There are no exact experimental data that would indicate the significance of lipoids and phosphatides.

Closely allied to fats are waxlike substances, similar to fats in their fatty consistency and solubility in ether. They represent complex esters of fatty acids and monatomic higher alcohols, more often of ceryl, cetyl, and mericyl alcohols, and likewise of a cyclic alcohol phytosterol. Waxlike substances are very

widespread in plants. Their chief significance is to increase the impermeability of the cell wall to water. For this reason, they are mostly found on the surface of stems, leaves, and fruits of plants, where they impregnate and cover the cuticle and cork tissue and increase their protective function against water losses.

Dibasic organic acids are very widespread in the cell sap of plants, both in a free state and in the form of salts. Those most frequently met with are oxalic acid, COOH·COOH; malic acid, COOH·CH_2·CHOH·COOH; and tartaric acid, COOH·CHOH. CHOH·COOH. Of the tribasic acids, the most widespread is citric acid.

$$\begin{array}{l} CH_2\cdot COOH \\ | \\ C(OH)\cdot COOH \\ | \\ CH_2\cdot COOH. \end{array}$$

Especially large amounts of acids are found in fleshy leaves of succulents, as well as in such plants as *Rumex* and *Oxalis*. Malic, citric, and tartaric acids are present in fruits, usually when not quite ripe. The presence of organic acids produces an acid reaction, which can be demonstrated in the expressed juice of most plants. In some plants, the actual acidity due to the presence of hydrogen ions may attain as much as pH 1.6 to 1.3. The exudate from phloem cells is frequently alkaline. Oxalic acid in the presence of calcium ions very often forms various-shaped crystals of calcium oxalate, such as druses, raphides, etc., described in detail in courses of plant histochemistry. Calcium oxalate crystals usually appear in cells that are approaching senescence; they are especially abundant in dying tissues. The oxalates seem to have a toxic action that may kill the cells in which they are depositing.

The formation of organic acids in plant tissue is closely associated with the process of respiration, and they may be regarded as by-products of oxidation processes. They are formed from pyruvic acid and acetaldehyde, which are intermediate products of the oxidation of sugars, which, with incomplete oxidation, form dibasic acids (Butkevich, Bernhauer). Such partial oxidation may be due to an insufficient access of oxygen. Organic acids accumulate chiefly in plants with thick leaves, succulents, as well as in the flesh of ripening fruit. In later stages of matur-

ity, the free acids combine with alcohols and carbohydrates to form complex esters which impart specific flavors to fruit.

12. Reserve Proteins and Products of Their Hydrolytic Decomposition.—The nitrogenous reserve substances of plants are represented almost exclusively by proteins. This group of compounds is of an extremely complex composition, consisting of at least five elements: carbon, oxygen, hydrogen, nitrogen, and sulphur and often phosphorus in addition. Nitrogen constitutes 15 to 19 per cent of the protein molecule. In many respects, protein substances are analogous to polysaccharides. They form large molecules of a definitely colloidal character and are

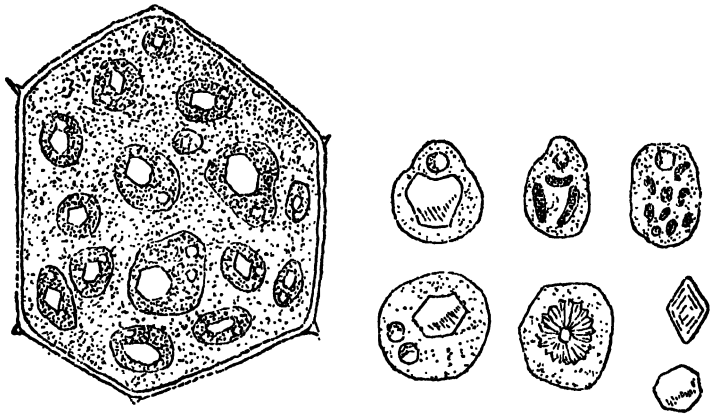


FIG. 13.—Cell from cotyledon of castor bean with aleurone grains. Individual aleurone grains with crystalloids at right.

comparatively easily hydrolyzed into more simple easily crystallizable products, which belong to the group of amino acids. The protein molecule, therefore, must be considered as consisting of a number of amino acids joined to each other through removal of water, just as starch or cellulose is composed of molecules of glucose united to each other. The essential difference, however, is that in splitting polysaccharides only one or two monosaccharides are formed, while at least 10 or more amino acids enter into the building of most protein molecules. Owing to this complex composition, the protein group is much larger than that of polysaccharides.

Reserve proteins usually are stored in seeds in the form of special bodies, called "aleurone grains." Essentially, they are dried-up vacuoles, very rich in soluble proteins (Fig. 13).

In many aleurone grains, it is possible to see distinctly well-defined crystals of protein substances, from which it is apparent that colloidal substances may have the capacity to crystallize. A more detailed description of the structure of aleurone grains may be found in books on plant anatomy. In underground food-storage organs, as in the tubers of the potato, reserve proteins are often found in a crystalline form.

Protein substances can be easily detected by the aid of color reactions, of which the most important is the biuret test. In this particular reaction, proteins give a blue-violet or red-violet color upon the addition of a solution of sodium hydroxide and a few drops of a weak solution of copper sulphate. Upon heating with strong nitric acid, proteins give a bright yellow color (xanthoproteic reaction), and upon boiling with mercuric nitrate in the presence of nitrous acid, a brick-red color (Millon's reaction).

It must be noted, however, that all these reactions, except the biuret, are tests not for the whole protein molecule but only for some of its more characteristic amino acids. These constituent amino acids may be studied best by means of hydrolytic decomposition of the protein molecule. For this purpose, the protein is subjected to a prolonged boiling with mineral acids, which hydrolyze it into its constituents. Almost always these are α-amino acids, in which NH_2 is linked to the same carbon atom to which is attached the COOH group. Otherwise, their atomic configuration is quite varied, being not only of the aliphatic but also of the aromatic series. All of them, with the exception of glycine (α-amino-acetic acid) are optically active.

The main amino acids found in proteins are the following:

A. Aliphatic series:

1. Monoamino acids:
   -Glycine, $CH_2NH_2{\cdot}COOH$
   d-Alanine, $CH_3CHNH_2{\cdot}COOH$
   l-Leucine, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!\diagdown\!\!\diagup CH{\cdot}CH_2{\cdot}CHNH_2{\cdot}COOH$
   l-Aspartic acid, $COOH{\cdot}CH_2{\cdot}CHNH_2{\cdot}COOH$
   d-Glutamic acid, $COOH{\cdot}CH_2CH_2CHNH_2{\cdot}COOH$.
2. Diamino acids:
   d-Lysine, $CH_2NH_2CH_2CH_2{\cdot}CH_2{\cdot}CHNH_2COOH$
   d-Arginine, $\begin{matrix}NH_2\\NH\end{matrix}\!\!\diagdown\!\!\diagup C{\cdot}NH{\cdot}CH_2{\cdot}CH_2CH_2CHNH_2{\cdot}COOH$
3. Oxyamino acids:
   l-Serine, $CH_2OH{\cdot}CHNH_2{\cdot}COOH$

4. Thioamino acids:
   l-Cystine, $COOH{\cdot}CHNH_2{\cdot}CH_2S{\cdot}SCH_2{\cdot}CHNH_2{\cdot}COOH$

B. Aromatic series:
1. *l*-Phenylalanine, $C_6H_5{\cdot}CH_2{\cdot}CHNH_2{\cdot}COOH$
2. *l*-Tyrosine, $C_6H_4OH{\cdot}CH_2{\cdot}CHNH_2{\cdot}COOH$

C. Heterocyclic compounds:
1. *l*-Proline, $(C_4H_7NH){\cdot}COOH$
2. *l*-Tryptophan, $(C_8H_5NH){\cdot}CH_2CHNH_2{\cdot}COOH$
3. *l*-Histidine, $(C_3H_2N_2H){\cdot}CH_2{\cdot}CHNH_2{\cdot}COOH$

A detailed study of proteins and their products of hydrolysis would be out of place here. Further discussion of this subject can be found in textbooks on organic or physiological chemistry. It should be pointed out, however, that it is the aromatic nucleus of tyrosine that gives the xanthoproteic and Millon's reactions. Cystine is the typical compound in the form of which sulphur enters into the composition of the protein molecule. From it is obtained hydrogen sulphide, which is always formed during the complete decomposition of proteins in the process of putrefaction. Among the products of the hydrolysis of proteins, besides amino acids, a small amount of ammonia and often carbohydrates also are found.

The percentage relationship of the amino acids varies in different proteins, and it is exactly these differences that are the basic cause of the enormous variety of protein substances. For example, the reserve proteins from the seeds of some agricultural plants contain the following quantities in percentage of the most important amino acids and ammonia.

	Legumin of peas	Phaseolin of kidney beans	Gliadin of wheat	Edestin of hemp
Alanine	2.08	1.80	2.00	3.60
Leucine	8.00	9.65	5.61	20.90
Proline	3.22	2.77	7.06	1.70
Phenylalanine	3.75	3.25	2.35	2.40
Tyrosine	1.55	2.84	1.20	2.13
Aspartic acid	5.30	5.24	0.58	4.50
Glutamic acid	16.97	14.54	37.33	14.07
Arginine	11.71	4.89	3.16	14.17
Lysine	4.98	4.58	0.0	1.65
Histidine	1.69	2.62	0.61	2.19
Ammonia	2.05	2.06	5.11	2.28

All of these amino acids are obtained from proteins through hydrolysis, apparently without any great changes in the structure of the amino acid molecule. This leads to the belief that they are linked to each other in such a way that the (acid) —COOH group of one unites with the (basic) —NH_2 group of another. It must be remembered that amino acids are amphoteric compounds, able to react as weak acids and as weak bases at the same time. As a result of such unions, there accumulates in the protein molecule a number of CO—NH—CH groups that are the ones that give the biuret test. An amino acid by itself will not give this reaction.

Basing his work upon these suppositions, Emil Fischer (1906) attempted to bring about a synthesis of the protein molecule. Binding together amino acids, first by twos, then by threes, and finally by as many as 18 or 19, he obtained complex compounds named by him polypeptides, which gave the biuret reaction and, in general, possessed many of the properties of proteins. The structure of the polypeptide may be represented by the following chain formula:

$$\begin{array}{l} —NH—CH—CO—NH—CH—CO—NH—CH—CO— \\ \qquad\quad | \qquad\qquad\quad | \qquad\qquad\quad | \\ \qquad\quad R_1 \qquad\qquad\quad R_2 \qquad\qquad\quad R_3 \end{array}$$

where R_1, R_2, etc., are parts of different amino acids, connected with the link CH of the main chain to form lateral chains. According to the theory of Emil Fischer, actual proteins represent nothing else than very complex polypeptides constructed in the same manner as his artificial polypeptides.

The investigations of Emil Fischer made an epoch in the study of proteins, and the preceding scheme for the structure of a protein molecule was for a long time generally accepted. Recent researches, however, have shown that besides such peptide bonds in the protein molecule, there also exist bonds that are not peptide linkages, leading to the formation of ring or cyclic structures, not open-chain compounds. From the demonstration of such cyclic compounds (Abderhalden and Karrer) it is assumed that the protein molecule contains bonds of a diketopiperazine character. Hence, the conclusion may be drawn that the protein molecule is of a much more complicated structure than it seemed directly after the brilliant work of Fischer.

It is assumed at present that the protein molecule is very large and that the molecular weight of protein substances may be several hundred thousand. Such a large size for the molecule explains the fact, observed long ago, that solutions of protein substances always possess a colloidal character and the boundary between molecules and micelles is effaced in them. The large molecules can be thrown out of a solution by high-speed centrifugation.

A reasonable classification of protein substances must be based on their composition, *i.e.*, on the quantitative relations of the amino acids composing them. Such a classification, however, has not yet been perfected, and it is customary to keep to the old order based chiefly on the different solubility of proteins in water and in salt solutions.

The main features of this classification may be stated thus. First of all, proteins are divided into two groups, the simple proteins and the complex proteins. The latter represent combination of a protein of the structure described above with some other complex molecule. If the latter is of a carbohydrate character, glucoproteids are obtained, which include certain mucous substances. If it is a complex of nucleic acids, nucleoproteins are obtained, which represent essential substances of the cell nucleus.

Simple proteins are divided into five groups:

1. Albumins—soluble in water.
2. Globulins—insoluble in water but soluble in weak solutions of neutral salts.
3. Prolamins—insoluble either in water or in salt solutions but soluble in 70 per cent alcohol.
4. Glutelins—insoluble in any of the foregoing solvents but soluble in dilute alkalies.
5. Histones—not found in a free state but in combination with nucleoproteins and other complex proteins.

Reserve proteins of seeds belong to the group of simple proteins. Of these, albumins are practically unknown in plants. Of the globulins, legumin and vicilin are found in the seeds of peas and some other legumes; phaseolin, in seeds of the kidney bean; conglutin, in lupine; and edestin, in hemp seeds. Of prolamins, the most important is gliadin, in wheat gluten; hordein, in barley; and zein, in maize. Of the glutelins, the

most common is glutenin, which forms part of the gluten of wheat.

Proteins represent the most important and most characteristic components of the living substance. They are absolutely indispensable for the formation of protoplasm, and the analysis of the protoplasm of a slime mold stated in Art. 9 shows that quantitatively likewise they form one of its most important parts. It would be erroneous to assume that life activity is entirely dependent on the structure of the protein molecule, but Stanley has recently shown that the crystallizable protein of viruses has many properties similar to living substance.

Lipoids and carbohydrates as well as electrolytes form a no less indispensable component of the living protoplasm. Life as a new quality, distinguishing organisms from lifeless bodies and creating a number of new essential features, is connected not with several separate groups of organic substances but with the entire complex forming the protoplasm. Protein substances isolated from this complex do not display all of the properties of living substance, of which a continuous metabolism is the most important. Similar to starch or fat, reserve proteins, like edestin or gliadin, when extracted from the cell represent only materials for the structure of living protoplasm.

13. Hydrolytic Decomposition of Reserve Carbohydrates and Fats during Germination of Seeds. Enzymes Accomplishing This Decomposition.—The group of substances examined above is capable of remaining permanently unchanged only in completely dry and dormant seeds. For this reason, seeds furnish a highly convenient material for obtaining and studying these substances. As soon as live seeds are moistened, a series of complex biochemical processes is initiated. These processes represent the initial stages of germination and permit the growth and development of the seed embryo by using the reserves stored in the cotyledons and other storage organs. The most characteristic feature of these processes is the decomposition of complicated storage substances into their components. Polysaccharides usually disintegrate into monosaccharides, fats into fatty acids and glycerin, and proteins into amino acids and ammonia. All these reactions of disintegration proceed with an addition of water and are of the general type of hydrolytic reactions.

To become acquainted with the course of hydrolysis in germinating seeds and living cells in general, it is necessary first to examine the case of hydrolysis of reserve carbohydrates, *viz.*, of starch. Microscopic observations have shown that from the first days of germination, starch undergoes an intensive chemical transformation. On the surface of the grains of starch, first appear small depressions, which gradually deepen into the grain. By the union of these depressions and the formation of cavities, the starch grain finally becomes so tunneled that it falls apart into minute granules, which in the end are completely dissolved (Fig. 14). In place of starch, there now appear sugars, *viz.*,

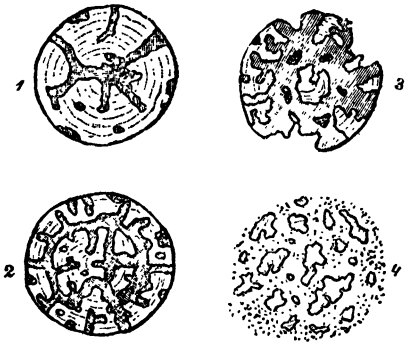


FIG. 14.—Successive stages in the direction of a starch grain by diastase (*after Benecke-Jost*).

maltose and glucose. It is easy to detect this accumulation of sugar by special reagents, such as Fehling's solution, or simply by tasting. The ungerminated grain of barley has a mealy taste, while the germinating one is sweet. Germinated barley after drying is called "malt" and is used extensively in brewing and other industries.

The germinating seed does not have at its disposal those agents, such as strong acids and high temperatures, by which the hydrolysis of polysaccharides is brought about in a laboratory. It does, however, have substances capable of bringing about this hydrolytic cleavage even at ordinary temperature. These substances are known as "enzymes." One of the first substances of this sort to be discovered in plants was the enzyme that hastens the decomposition of starch, known as "diastase" or "amylase."

Diastase can be easily obtained from germinating grain, or malt. The finely ground dry malt is soaked in water, the resulting decoction is filtered, and the filtrate then precipitated with alcohol. The white, flaky precipitate is filtered off and again dissolved in water. When added to a starch paste, this solution will hydrolyze it rapidly. After a short time, the blue iodine coloring characteristic of starch begins to change, first to violet, then to red, then to a yellowish color; and finally, the solution ceases to give the iodine reaction. With Fehling's solution, it will, however, show a distinct test for sugar. A detailed chemical analysis will show that instead of starch, maltose is present.

The action of diastase, accordingly, differs somewhat from the behavior of inorganic catalysts, such as strong acids, which attack the molecule, or, more exactly, the colloidal aggregate of starch, and rapidly break it up into the end product of hydrolysis, glucose. Whereas diastase acts more slowly, as is indicated by the gradual disappearance of the color reaction with iodine, the whole process usually stops at the maltose stage. Diastase reduces step by step the colloidal aggregate that is called starch; in other words, it increases the degree of its dispersion. It produces first the intermediate products of the breaking-up process, the dextrines, and then maltose, when its action ceases. It is not able to split maltose into glucose.

Besides its gradual action, diastase differs from inorganic catalysts also in its specificity. It can act only upon starch and produces no effect on the reserve celluloses, which are hydrolyzed by acids with no greater difficulty than is starch. Likewise, it has no effect on either inulin or cane sugar.

Only a very small amount of diastase is present in dormant seeds. During the early stages of germination, therefore, the decomposition of starch proceeds very slowly. At the time of swelling, however, the quantity of diastase in the seed increases noticeably, and during the first few days of germination it shows a marked increase. At the end of the first week of germination, the amount of diastase is usually three to four times as great as it was at the completion of swelling.

This increase of diastase during the progress of germination takes place only in the presence of a sufficient supply of oxygen and is most closely connected with the very active respiration

shown by germinating seeds. If the seeds are deprived of oxygen, the accumulation of diastase ceases at once.

In the grain of cereals, such as wheat and corn, the centers of formation of diastase are predominantly the embryo of the seed, especially its scutellum (Fig. 15) and the aleurone layer surrounding the endosperm. Thence it diffuses into the tissue of the endosperm, causing decomposition of the starch stored there. It is interesting to note that the conversion of starch into sugar in the endosperm proceeds to completion only if the starch remains in close contact with the scutellum and the young sprout adjacent to it, which continually absorbs and utilizes the sugar formed during the hydrolysis of starch. If these parts are removed, the process of transformation of starch in the endosperm stops quickly as a result of the accumulation of the soluble end products of the reaction.

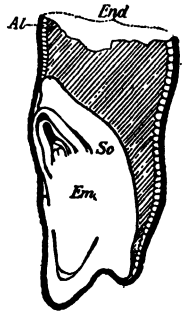


FIG. 15.—Longitudinal section through the lower portion of a grain of wheat (diagrammatical). *End*—endosperm, *Al*—aleurone layer, *Em*—embryo, *Sc*—scutellum (*after Benecke-Jost*).

Simultaneously with the decomposition of starch, the cell walls of the endosperm also are dissolved at germination. Formerly, it was supposed that this was accomplished by diastase. More detailed research has proved, however, that another enzyme, cytase, is at work here, which can be obtained in a comparatively pure state from the germinating seeds of lupine or the seeds of the date palm. Like diastase, cytase is formed largely by the embryo. The dissolving of the thickened walls of the endosperm of the date palm stops when the embryo is removed.

Besides these polysaccharide-splitting enzymes, there are others at work during germination that hydrolyze the disaccharides and glucosides. Thus, in the conversion of starch into sugar, along with diastase, often maltase is present, which changes maltose into 2 molecules of glucose. Another very widely distributed and much studied enzyme is invertase, which splits cane sugar into glucose and fructose by a process called "inversion." It is especially easy to obtain invertase from yeast, the presence of which leads to hydrolysis of sucrose which otherwise is not fermentable. When yeast is triturated with pure quartz sand and a small quantity of warm water and

filtered, a transparent filtrate showing a strong invertase action will be obtained. After precipitation by alcohol, a dry preparation of invertase readily soluble in water and preserving its action for a rather long time is secured. Of the enzymes that split glucosides, the most important is emulsin, or β-glucosidase, which hydrolyzes all the glucosides of the β-group. It is usually obtained from seeds of bitter almonds. The enzyme α-glucosidase, closely related to it, acts in the same manner on α-glucosides. It is found along with invertase in the extract from yeast.

In oily seeds, the main nonnitrogenous reserve substances are represented by plant fats.

In the germination of oily seeds, the stored fat is used very rapidly. Müntz observed that of 8.9 g. of fat found in 20 g. of poppy seeds prior to their germination, after 2 days there were left 6.8 g. and after 4 days only 3.9 g. This consumption of oil is preceded by its hydrolysis, which may be seen from the fact that the quantity of free fatty acids, which is quite negligible in the seeds, grows very rapidly at germination. In the case of the germinating poppy seeds, only 10 per cent of the fat was composed of free fatty acids before germination, but after 2 days this had increased to 53 per cent, and after 4 days to 97 per cent. It was not possible to demonstrate an accumulation of glycerin, as it very quickly undergoes further changes.

The hydrolysis of fats in germinating seeds is brought about by special enzymes, known under the general name of "lipases." Lipase is easily obtained by grinding with glycerin germinating castor beans. It will rapidly hydrolyze fats into glycerin and fatty acids. Since the rate of the reaction is considerably increased in an acid medium (pH 5) and since, in hydrolysis, free acids accumulate, the activity of lipase has an autocatalytic character. In the beginning, it proceeds rather slowly and then with ever increasing rapidity.

14. Decomposition of Reserve Proteins during Germination. Proteolytic Enzymes.—Reserve proteins of the seeds are also subject to hydrolysis during germination. This is brought about by proteolytic enzymes, or proteases. The result of the activity of these enzymes is the accumulation of amino acids. According to the analysis of Jodidi (1925), 95 per cent of the total nitrogen substances in the grain of corn are in the form of proteins. Two days after the beginning of germination the amount of nitrogen

fell to 78 per cent, 4 days later to 61, and at the end of 6 days to 57 per cent with a corresponding increase in the quantity of peptids and amino acids.

The first of the proteolytic enzymes to be studied were those present in the digestive tract of animals. Two types were established: pepsin, secreted by the walls of the stomach and causing a rather weak hydrolysis of proteins into the so-called "peptones"; and trypsin, secreted by the pancreas and splitting proteins and peptones into amino acids. Peptones are quite complex bodies of a colloidal nature, but their molecular weight is less than that of the true proteins.

It is usually thought that peptones are the first products of the decomposition of the protein molecule; but recently evidence has accumulated which shows that in the formation of peptones the protein molecule itself is not decomposed, but only the larger colloidal aggregates, composed of several molecules, are broken down. At any rate, peptones do not represent definite chemical compounds. In their chemical composition and properties, they are most closely allied to polypeptides, obtained by the method of Emil Fischer by combining several amino acids.

Further decomposition of peptones is accomplished by trypsin, as well as by another special enzyme, erepsin, which is distinguished by its inability to hydrolyze unchanged proteins. Thus proteins are hydrolyzed in the digestive tract of animals by three enzymes: pepsin, trypsin, and erepsin, which supplement each other in their activity.

In plants, proteolytic enzymes were discovered much later than in animals, and until recently they were comparatively little studied. The best known of the active plant proteases is papain, obtained from the juice of the fruit of *Carica papaya*. It is used by persons suffering from indigestion due to insufficient secretion of proteases. Extraction of proteases from germinating seeds is more difficult than the extraction of diastase, for they are not soluble in water. Their activity is usually studied through autolysis, *i.e.*, "self-digestion" of protein substances of the seed. The seeds are ground to flour, and water is added in the presence of chloroform, toluol, or some other neutral antiseptic, which precludes the development of microorganisms during the digestion and likewise kills the cells of the seed embryo that may have remained unbroken during the grinding.

Investigations in recent years, especially those of a most eminent authority in biochemistry, Richard Willstätter, and his followers, have produced great changes in our ideas concerning proteolytic enzymes. At present, they are divided into two large groups: proteinases, affecting unchanged colloidal protein substances; and ereptases, the activity of which is restricted to the decomposition of peptones and polypeptides. Proteinases in their turn are divided into three groups: (1) pepsinases, active in a highly acid medium (pH = 2) (this is chiefly pepsin of the stomach juice); (2) enzymes, such as papain, active in a slightly acid or neutral medium (pH = 4 to 7); and (3) enzymes, like tryptases, active in an alkaline medium (pH = 8 to 9). This division corresponds to principal differences in the conditions of their activity. Pepsin decomposes cations of protein formed in an acid medium; trypsin, anions of protein formed in an alkaline medium; and papain decomposes isoelectric proteins.

Peptase and tryptase are found chiefly in animals in the secretions of the digestive glands and are active outside the cells in the digestive tract. In plants, they are found only in exceptional cases, *e.g.*, in insectivorous plants that catch and digest their prey in special organs or in traplike leaves that secrete a digestive juice from special glands. In most cases, proteolytic enzymes of plants are active inside the cell and are not capable of diffusing into the surrounding medium. For a long time, these enzymes were grouped with tryptases, but recently it has been made clear that they belong to the group of papain. The plant ereptases must also be referred to the group of intracellular enzymes. The animal organism possesses an enzyme corresponding to the plant papain, *viz.*, catepsin, which is likewise active inside the cell.

15. General Properties of Enzymes. Methods of Their Isolation and Purification.—Enzymes are usually defined as organic catalysts produced by living cells but capable of manifesting their activity outside of the cell and acting on specific substances.

The term "catalysts" designates substances that do not enter into chemical reactions as reactants but accelerate the reactions by their physical properties. They are not found in the end products of the reaction, or consumed in the reaction, though their participation in intermediate reactions is not excluded and

sometimes seems very probable. Spongy platinum represents a typical inorganic catalyst. In the process of manufacturing sulphuric acid, spongy platinum may hasten the oxidation of sulphur from the sulphurous to the sulphuric state by offering a suitable surface on which the sulphur dioxide and oxygen can condense in concentrations high enough to speed up their reaction. The platinum black is not used up in this reaction.

Enzymes are considered organic catalysts of a colloidal nature. Because of their dispersed condition, they possess a large adsorbing surface and therefore considerably speed up a reaction that is going on in their presence. Being colloidal substances, enzymes are unstable in many ways. They coagulate when boiled, thus losing their activity. Solutions of enzymes, if left to stand for a long time, likewise lose their activity, in all probability owing to the gradual decomposition or denaturation of the enzyme. They are easily precipitated in water solutions by various dehydrating substances, such as alcohol or acetone, but may be redissolved.

Many substances highly toxic to living protoplasm, *e.g.*, thymol, chloroform, toluol, and others, do not affect harmfully the action of the enzymes; that is why these antiseptics are usually added to solutions of organic substances in studying enzymatic processes, for the purpose of precluding the development of putrefactive and fermentative microorganisms. Enzymes are thus chemically considerably more stable than the protoplasm that produces them. But many substances, like salts of heavy metals, formaldehyde, and other toxic substances, will destroy enzymes by causing an irreversible precipitation and a loss of their function.

One of the greatest difficulties to be overcome is the question of the chemical composition and nature of enzymes. The usual methods of precipitation by alcohol or acetone are the same as methods used for precipitating protein substances, with which they usually precipitate. Because of this behavior, enzymes were for quite a while classified with the protein group. But careful investigations long ago created doubts as to the protein nature of enzymes and led to the suggestion that the general behavior in precipitation is due to coagulated proteins carrying along with them the enzymes, just as they are known to carry along dyes of a colloidal nature, fine sediments, etc.

As a result of the investigations by Willstätter and his colleagues, it has been possible during the past few years to understand somewhat more clearly the chemical nature of enzymes. Willstätter has based his work largely on the method of selective adsorption of enzymes by certain finely ground substances, such as clay and kaolin. Owing to their extremely high adsorptive capacity, enzymes are removed from solutions more readily by these substances than proteins, resins, or other colloids. By repeated dissolving and adsorption, it is possible in this manner to concentrate certain enzymes and to purify them from various admixtures. The best results are secured with invertase from yeast, which may be extracted in a quite pure condition by water. Willstätter was able to increase the concentration of this enzyme 1,600 times. He has shown that such an almost pure preparation of invertase does not contain phosphorus and does not give a reaction for proteins or carbohydrates. But after all, a preparation of this type is not absolutely pure, and an analysis of it does not give a true idea of its chemical nature.

Purified enzymes are unstable, and under usual conditions the molecules of enzymes are nearly always adsorbed on colloidal particles. Willstätter has given these particles the name of "colloidal carriers." A considerable part of the physicochemical peculiarities of solutions of enzymes is determined by the properties of the carriers. Complete separation of the enzyme and its carrier is difficult to attain, and the numerous contradictions and enormous accumulated literature about enzymes may be explained by the fact that investigators studied the properties not so much of enzymes as of their colloidal carriers. A number of enzymes have now been obtained in crystalline form by Semper, Northrop, and others. These seem to be the pure substances free from admixture.

Because it is not possible to isolate certain enzymes in a completely pure state, it is not possible to measure them quantitatively. The fact of their presence is established by their activity, and their amount by the quantity of substances transformed per unit of time. However, even in applying crude unpurified preparations of enzymes, one of their most interesting properties was ascertained, *viz.*, their capacity of transforming enormous amounts of organic substances, even when the enzyme is taken in the minutest quantities. This property is still more

evident when purified preparations are used. For instance, 1 gm. of invertase can hydrolyze a whole ton of sucrose, a quantity one million times its own weight. It must not be concluded, however, that the quantity of enzyme is of no importance. On the contrary, if the velocity of hydrolysis is taken into account, the latter is found to be in direct proportion to the amount of enzyme, provided that there is a sufficient amount of substrate. Therefore, if rapid disintegration of a substance is desired, large quantities of the enzyme must be applied.

The question of the reversibility of enzymatic action is of great importance and interest. According to the general laws of physical chemistry, catalysts, accelerating the course of a reaction, do not, as a rule, change the points of equilibrium between the initial and the end products. But the reactions of hydrolysis are, generally speaking, reversible, *i.e.*, in the case of a large number of products.

Hydrolysis and the introduction of the elements of water into the reaction products may be reversed in condensation and the removal of water. Theoretically, at least, it should be expected that the same enzymes will accelerate both the decomposition and the synthesis of a complex reserve substance in the organism. In the majority of enzymatic reactions, however, this does not hold true. In a case in which it was possible to obtain synthesis by the aid of an enzyme, the product was found to be an isomer of the initial substance. From a concentrated solution of glucose with the aid of maltase, Croft Hill (1898), for instance, obtained not maltose, but isomaltose. Recently, however, Bourquelot was successful in synthesizing with the aid of enzymes a large number of glucosides from the products of their decomposition, and in such a way that the possibility of enzymatic synthesis was irrefutably demonstrated. It can be assumed with a great degree of probability, therefore, that synthesis of polysaccharides, fats, and proteins, which are so common in plant cells, proceed under the influence of the same enzymes that cause their decomposition. The conditions under which such enzymatic syntheses take place are not as yet fully known. It seems to be certain, however, that the continuous removal of water from the reaction is necessary. Syntheses of this kind most probably take place in maturing seeds, where the reserve substances are found. The hydrolytic

products are produced during the soaking and germination of seeds.

Enzymes represent the most important chemical reagents of the cell. Judging from a number of properties, especially from their easy destruction by heat and toxic substances, enzymes must be closely related to living protoplasm. Still, considering the ease with which they may be dissolved and precipitated, one is forced to classify them with nonliving substances. With the aid of more refined methods of killing, such as desiccation, freezing, or the action of antiseptics, such as chloroform, it is possible to kill the cells of plants without destroying the enzymes found within them. According to Palladin, plants treated by one or another of these methods are killed. They should be distinguished from plants that have died as a result, for example, of boiling, in which not only the plasma is killed but all its enzymes.

In carefully killed plants, all the enzymes present in their cells continue to function for a time, and therefore this method is very often used in the study of enzyme activity. However, very soon there are revealed in such cells many irregularities and degressions from ordinary enzymatic action, and many of them will cease to work altogether. This probably happens because in a dead cell coordination between the activities of the various enzymes is lost. Often some of them begin to destroy others; the reactions in the cell medium exercise a very great influence upon the whole course of digestion. This phenomenon is called "autolysis" or "autodigestion of the cells," in which the proteolytic enzymes usually continue to work longer than the others.

It is not known in just what way the coordination of the work of the enzymes is brought about in the living cell. According to Oparin (1934), an important role belongs to the phenomenon of adsorption of enzymes by colloids, especially by protein substances. With the continuous alternation of the processes of coagulation and dispersion of colloids in the living cell, at times the activity of some of the enzymes weakens or stops completely; with coagulation, at others, on the contrary, it increases, with the dispersion of the colloids. Apparently there is always in the cell a reserve of enzymes that are firmly adsorbed by colloidal substances. Only a certain part of this reserve is active, momentarily going into solution.

16. Influence of the Condition of the Medium upon the Activity of Enzymes. Specificity of Function of Enzymes and Their Classification.—The condition of the medium greatly affects the activity of enzymes. The same enzyme may exhibit a high degree of activity under certain conditions, while under others it may be entirely inactive.

Of the external conditions that exert a great influence on the rate of enzymatic reactions, temperature must be considered of primary importance. The effect of temperature on the activity of an enzyme has much in common with its influence on vital processes. With an increase of temperature, the reaction first accelerates; then a certain optimum temperature is reached at which the reaction proceeds at its highest rate. With a further increase of temperature, the reaction begins to decrease; and, finally, the destruction of the enzyme occurs. In short, on the temperature curve of enzymatic action there are three cardinal points characteristic of vital processes: the minimal point, at which the reaction only begins to show a perceptible activity; the optimal point, at which it proceeds at its highest rate; and, finally, the maximal point, at which the reaction ceases. (For a more detailed discussion of cardinal points, see Art. 30.) In distinguishing enzymes from the living protoplasm, the fact should be noted that their optimal points lie considerably higher, *i.e.*, at 45 to 50°C. and in some enzymes even at 60°C. The optimal for living cells usually does not exceed 25 to 35°C., while a temperature of 45 to 50°C. is usually lethal. The complete destruction of the enzymes is attained at a still higher temperature. The usual method of stopping enzymatic action is by boiling.

The activity of enzymes is likewise determined to a high degree by physicochemical factors of the medium, especially by the hydrogen-ion concentration. In examining the proteolytic enzymes, the fact has already been emphasized that each group works only at definite pH values and outside these limits becomes inactive. For other enzymes likewise, definite optimal pH values have been determined at which they display their greatest activity. For amylase (diastase) of malt, this pH will be about 4.6 to 5.2; for maltase, 6.1 to 6.8; for invertase (sucrase), 4.2; etc. However, it should be observed that these magnitudes of pH are not absolute. They may change considerably under

the influence of different admixtures, of which it is most difficult to purify the enzyme solutions, as well as in dependence upon the colloidal nature of the substances associated with the enzyme.

Of great physiological significance is the change of the activity of enzymes under the influence of various kinds of "activators" and "paralyzers." These designations are given to substances that, being added sometimes in the slightest quantities, exert a great influence upon the work of enzymes. An especially thorough study has been made of the activators of proteolytic enzymes, and it has been proved that different groups of proteases are activated by different substances. Thus, enzymes of the group of papain are activated by hydrocyanic acid and hydrogen sulfide, in particular by natural compounds including the sulphydryl group SH, the most important of which are glutathione and cysteine, that are liberated in the process of hydrolysis of protein substances. Tryptases are activated by a special activator enterokinase, first demonstrated by Pavlov. It is secreted by the mucous membrane of the intestine, but its nature has not yet been ascertained. HCN, H_2S, and glutathione do not activate tryptase; on the contrary, they suppress its action. For the group of peptases, no activators are as yet known.

Closely allied to the activators are the so-called "coenzymes," the presence of which is thought by some authors to be quite indispensable for certain enzymatic reactions. The action of the coenzyme is very often represented in the following manner: the coenzyme reacts with the initial substance, and this complex is then subject to the action of the enzyme. The enzyme is not capable of acting upon the initial substance when in its pure form but only after it has become combined with the coenzyme. Antienzymes, on the contrary, inhibit the activity of enzymes, transforming the initial substances into compounds that are unavailable to the enzyme. About the action of coenzymes and antienzymes, however, very little has thus far been ascertained, and the distinctions between them and activators and paralyzers is not yet established. Some authors, as, for instance, Kostytchev, completely deny the existence of coenzymes and antienzymes.

A very characteristic peculiarity of enzymes is the specificity of their action. It has been shown that starch is converted into

maltose only by diastase and cellulose only by cytase, and that the hydrolysis of two disaccharides, maltose and sucrose, was brought about, respectively, by maltase and sucrase. The classification and nomenclature of enzymes are based on this selective action. In giving the enzyme a name, the name of the substance it hydrolyzes is taken with the addition of the suffix "-ase." Some of the enzymes found and described in earlier times retain their historical names, *e.g.*, diastase, invertase, pepsin, and several others.

The specificity of enzymatic action should not be understood in the sense that each disaccharide or polysaccharide, each protein, each fat, etc., can be hydrolyzed only by a special enzyme. Quite the contrary; it is established beyond doubt that there are fewer enzymes than are often described, and that various proteins, for example, are acted upon by one and the same enzyme. Their specific action consists largely in the fact that each enzyme possesses the ability to break down any linkage of a certain character in the complex molecule of organic compounds and that in the presence of two optical isomers, each enzyme may possess the ability to act only upon one of them.

Since little is known of the chemical composition of enzymes and since their presence is attested to only by their activity, the classification of enzymes is thus far based on the classification of enzymatic reactions. Up to the present time, there is no well-established, unified classification of enzymes, and different authors adhere to different systems. The most consecutive classification of enzymes is the one suggested recently by Neuberg and Oppenheimer. According to this classification, all the enzymes are first of all divided into two groups, hydrolases and desmolases. Hydrolases catalyze hydrolytic processes, *i.e.*, processes where there is a rupture of the linkages between atoms of carbon on the one side and either oxygen or nitrogen on the other with the introduction of water at the point of the break. Desmolases represent enzymes that break the linkages of the chains of carbon atoms, *i.e.*, cause the so-called "desmolysis."

Each of these two groups is in its turn divided into two subgroups. The group of hydrolases includes all the enzymes that have thus far been examined, *viz.*: (1) *carbohydrases* disintegrating complicated carbohydrates, disaccharides (invertase, maltase), and polysaccharides (diastase, cytase, and others); (2) *amidases*

disintegrating protein substances (papain, peptase, tryptase, and others) as well as other compounds, the molecules of which possess the same type of connections as proteins (urease acting on urea, arginase decomposing arginin, and others); (3) *glucosidases* hydrolyzing glucosides; (4) esterases decomposing compounds having ester linkages (lipase); and (5) several other enzymes.

The most important representatives of the group of desmolases are the enzymes by means of which the processes of respiration and fermentation are activated. These processes represent sources of free energy, indispensable for the vital activity of organisms. They will be further discussed when these problems are being examined in Chap. IV.

17. Synthetic Processes during Germination. The Utilization of Carbohydrates and Fats in Germinating Seeds.—The metabolism of organic substances during the germination of seeds does not terminate with the formation of the final products of hydrolysis, *viz.*, simple sugars from starch and other polysaccharides, glycerin and fatty acids from fats, and mixtures of different amino acids from protein substances. All these processes of disintegration further pass into processes of synthesis; for the phenomenon of germination represents not only an expenditure of accumulated reserves but also the formation of new, vitally active organs. The interrelations of disintegration and synthesis are manifested by the gradual emptying of those parts of the seed that serve as storage organs, *viz.*, the cotyledons and endosperm, accompanied by their gradual shrinkage and desiccation, and on the other hand by the rapid increase in size of the parts of the embryo and the initiation of the vegetative organs of the young plant, its roots, stems, and leaves. In order to isolate the digestion processes taking place during germination for the purpose of studying them, the germinating seeds are usually killed so as to preserve the enzymatic system. This is achieved by applying autolysis: the soaked and finely ground seeds are placed in an antiseptic solution such as chloroform or toluol that does not affect the activity of the enzymes (see Art. 15). The study of synthetic processes during germination represents a far more difficult problem, for it is not possible to create such conditions as are normal in synthesis. For this reason, the synthetic processes in germinating seeds can be followed only by comparing the changes in chemical composition

during germination with those occurring during autolysis. Such a comparison, however, does not always afford a sufficiently clear picture; for the processes of hydrolysis and synthesis during germination are complicated by the intense oxidation of organic substances due to respiration. For this reason, information concerning the synthetic processes in germinating seeds is less exact and definite than is that concerning the processes of hydrolytic disintegration of protein substances.

The clearest concept of the changes of a synthetic character during germination has been obtained for carbohydrates. The chief substance utilized in the process of respiration during germination is glucose, the sugar most frequently found in plants and representing the final product of hydrolysis of more complex carbohydrates. During the germination of seeds in darkness, the chief nitrogen-free reserve substances are starch or other polysaccharides, which rapidly decrease in amount. For instance, according to the observations of Yokum, 3 days after the beginning of germination, 20 per cent of the carbohydrates disappeared from seeds; 6 days later, 35 per cent; 9 days later, 63 per cent; and 12 days after germination, 81 per cent. But if the fate of the separate groups of carbohydrates is followed more closely, it will be found that the most rapid decrease occurs in starch, which disappears entirely a few days after germination. The amount of glucose and monosaccharides in general first increases rapidly, then diminishes; while the amount of cellulose increases continuously. This is a consequence of the development of new organs accompanied by an increase in the number of cells and a corresponding increase in the mass of cell walls.

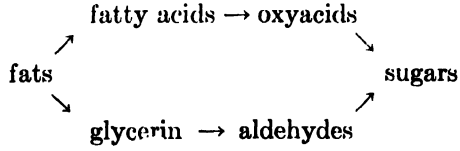
Simultaneously with the transformation of carbohydrates during germination, there is a change in the activity of the hydrolyzing enzymes. The quantitative determinations of Bach and Oparin have shown that if the activity of amylase (diastase) in dormant seeds is expressed by the value 1, 3 days after the beginning of germination it will be equal to 3; 6 days later, 20; and 8 days later, 23. Up to the time when the starch is almost completely dissolved, the activity of diastase increases more than twenty times. But at present, it cannot be definitely stated whether there is an actual increase in the quantity of enzyme, or whether it is the reserve enzyme, activated some-

how during the process of germination, that causes increased hydrolysis.

The metabolism of fats is more complicated than the transformations of carbohydrates. The intensive hydrolysis of fats does not lead to any considerable accumulation of fatty acids and glycerin. These products of the transformation of fats very soon undergo further transformations, and sugars appear in their place. In studying the germination of sunflower seeds, Miller (1910) observed the following chemical changes in their composition. One hundred originally dormant seeds contained 4.0 g. of fats and 0.3 g. of sugars. Four days after germination began, they contained 3.2 g. of fats and fatty acids and 0.08 g. of sugars. On the seventh day after germination, there were 1.5 g. of fats and 0.55 g. of sugar. Seven days later, there were 0.55 g. of fats and 0.21 g. of sugars. From these data, it is apparent that the quantity of sugar diminishes rapidly in the beginning of germination owing to an intense sugar utilization; later on, it increases again on account of the rapidly decreasing amounts of fats. Toward the end of germination, over 80 per cent of fats had disappeared, and the amount of sugar began to decrease again. Glycerin is not found in any considerable quantities, for it is rapidly transformed into other products.

The transformation of fatty acids into sugars in germinating seeds proceeds with the same ease as the reverse process of the formation of fats on account of carbohydrate translocation from the leaves into the ripening seeds. However, these changes have not yet been accomplished artificially, and their chemical nature is not clearly understood as yet. It can only be pointed out that the transformation of fats into sugars is essentially a process of oxidation and is accompanied by the absorption of oxygen.

According to the investigations of S. L. Ivanov, the transformation may be represented in the following diagram:



It is of interest to note that in spite of the ease with which this process takes place in plant and animal organisms, it is most

difficult to reproduce under artificial conditions, where it requires many complex synthetic reactions.

18. Metabolism of Proteins during Seed Germination. Processes of Decomposition and Synthesis. The Significance of Ammonia and Asparagine. A General Outline of the Metabolism of Reserve Substances during Germination.—As has been seen in Art. 15, the metabolism of protein substances during germination begins with an intensive decomposition of the reserve proteins and with the accumulation of easily soluble crystalline products of their decomposition, the amino acids.

It is especially convenient to follow the degradation of proteins in seeds germinated in the dark, for in this case the processes of synthesis that take place in light are inhibited. By this method, it was proved that over two-thirds of the total protein is transformed to more simple products in spite of the fact that germination even in the dark is accompanied by growth of the seedlings, a considerable increase of the number of living cells, and consequently an increase of the total amount of living protoplasm. Prianishnikov found (1895) in one of his experiments on the germination of vetch seeds in the dark that 10 days after the beginning of germination, there remained only 50 per cent of protein substances undecomposed; 10 days later, 40 per cent; another 10 days later, 33 per cent; and finally, 40 days after germination, when the seedlings were approaching death from exhaustion, there remained 32 per cent. These figures show that, in spite of extreme starvation, the protoplasm persistently retains a part of the protein substances as such. The reserve proteins of the seed were of course completely decomposed by that time. The protein substances that have not disintegrated with starvation belong chiefly to the group of nucleoproteins, complex proteins representing compounds of simple proteins with nuclein.

Corresponding with this intensive disintegration of proteins during germination, there is a rapid increase of the activity of the proteolytic enzymes. According to the data of Bach and Oparin (1923), on the third day of germination, the activity of protease is 6 times its value in dormant seeds; on the sixth day, 30 times; and on the eighth day, 45 times its initial value. Later on, the activity of protease slows down somewhat.

The physiological significance of the splitting of proteins during the germination of seeds consists in the fact that these proteins first of all must be transformed into a conveniently mobile form, since they must be transported from the reserve tissues, such as the endosperm and the cotyledons, into the growing parts of the developing embryo. Moreover, reserve proteins are only slightly soluble or entirely insoluble; and, even in a dissolved form, they are unable to diffuse through the cell membranes, owing to their colloidal nature. Another no less important result of the breaking down of proteins into amino acids is their loss of specific nature. Every protein being characterized by a combination of amino acids specific to it, it is not possible therefore to change one protein directly into another. In order to do this, it is necessary first to decompose it into its simplest integral parts, and only then may they be combined into a new protein molecule. This is attained by a complete destruction of the protein molecule into the end products of its hydrolysis.

A part of the amino acids formed during disintegration of the reserve proteins is not directly utilized in the construction of new protein molecules on account of the differences in the composition of proteins stated above. These "superfluous" amino acids disintegrate further into ammonia and nitrogen-free substances by means of the activity of a special enzyme desamidase. That is why ammonia is always found among the products of disintegration in germinating seeds. It never accumulates in significant quantities, however, for immediately after this last stage of decomposition of proteins is attained in the seedling, synthetic processes commence, which greatly complicate the general picture of the metabolism of nitrogenous substances during germination. Boussingault (1864) has pointed out the fact that in germinating seeds, especially in legumes rich in reserve proteins, there accumulates a large quantity of asparagine, an amide of aspartic or aminosuccinic acid,

$$CONH_2—CH_2—CHNH_2—COOH.$$

He expressed the idea that asparagine is analogous to the amide that accumulates in the blood of animals, *viz.*, urea, NH_2CONH_2. The production of asparagine is a way of fixing

ammonia, the accumulation of which would expose the organism to the danger of being poisoned with this toxic product.

Boussingault's idea has been substantiated experimentally through the work of Schulze (1875), who showed that, in reality, asparagine is not the initial product of the decomposing protein molecule, since there accumulates much more of it in seeds than there was of aspartic acid in the reserve proteins. The increase of asparagine, especially during the later stages of germination, is at the expense of certain of the remaining amino acids. By comparing the analysis of sprouts of peas 1 week old with those 3 weeks old, Schulze found the following quantitative changes of the most important amino acids and asparagine:

	Leucine	Tyrosine	Arginine	Asparagine
First week	Much	Little	Trace	None
Third week	Much less	Disappeared	Almost disappeared	Very much

The amount of asparagine accumulating in the seedlings may constitute up to 60 to 70 per cent of the total amount of protein found in the seeds before germination. The highest percentage of aspartic and the related glutamic acids does not exceed 20 to 25 per cent.

Asparagine, accordingly, is not the initial product of hydrolysis, but the result of union of nitrogenous substances with a carbon chain. This view received a final substantiation through the experiments of Prianishnikov. On germinating seeds in solutions of ammonium salts, he observed the synthesis of asparagine in seeds not only from the amino acids present during germination but also from ammonia supplied from without. In this connection, it is interesting to note that not all seeds bring about this synthesis with equal ease. It proceeds readily in seeds rich in nonnitrogenous reserves, such as the carbohydrates and fats, *e.g.*, in the seeds of graminaceous plants. In seeds of leguminous plants, in which there are large amounts of reserve proteins but in which the carbohydrate content is comparatively small, asparagine can be synthesized from ammonium salts only when calcium carbonate is supplied at the same time, which neutralizes the free acid. But the seeds of lupine, which are very

or in nonnitrogenous reserves, are unable to form asparagine
om ammonia, since the necessary carbohydrates are lacking.
here may even be observed a decrease in the synthesis of
paragine in the presence of ammonium salts. But if the seeds
lupine are given carbohydrates, one of the best methods of
ing which is to germinate them in light, they may display
intense synthesis of asparagine in the presence of ammonium
loride, in other words, with ammonia presented from sources
tside of the seed.

Prianishnikov illustrated the interrelations between the protein
bstances, asparagine, and ammonia in the following diagram:

$$\begin{array}{ccccc} \text{amino acids} & \rightarrow & \text{proteins} & \rightarrow & \text{amino acids} \\ \uparrow & & & & \downarrow \\ NH_3 & \leftarrow & \text{asparagine} & \leftarrow & NH_3 \end{array}$$

The left side of this diagram gives a picture of the synthesis
proteins in plants from the products of disintegration. The
ht side represents the course of disintegration. This diagram
es a very clear picture of the unity of two opposite processes
decomposition and synthesis of proteins and the role of
paragine as a connecting link between decomposition and
nthesis.

According to the picturesque expression of Prianishnikov, NH_3
ust be considered the alpha and omega of the metabolism of
otein substances in plants. It is liberated from amino acids
d represents the final product of disintegration of nitrogenous
bstances. But it represents likewise the initial substance with
hich there starts the synthesis of nitrogenous compounds from
rogen-free substances, the first product of this synthesis being
paragine.

In some plants, especially in fungi, that are rich in proteins
d poor in nitrogen-free substances, the intermediate product
the protein metabolism, asparagine, is replaced by urea. In
is respect, they function somewhat like the animal organism.
pecially large amounts of urea are accumulated in the fruiting
dies of *Lycoperdon*, in which it constitutes 10 per cent or more
the total dry weight. In plants whose cells are very rich in
ganic acids, the ammonium salts of these acids are accumulated
stead of asparagine.

This analogy between asparagine and urea emphasizes a very
portant physiological difference between animals and plants.

Animals fix the nitrogen, resulting from the complete disintegration of proteins, in the form of urea and excrete it from their bodies as unnecessary refuse. It is of no use to the animal organism, which cannot bring about the reverse synthesis of amino acids and proteins from ammonia and nonnitrogenous substances. Plants on the contrary, having high synthetic capacity, fix ammonia in the form of asparagine, which remains unaltered in the plant and then serves as the foundation material for further synthesis, splitting off ammonia when needed. Asparagine is the initial product of the synthesis that always accompanies the hydrolysis of protein substances at germination. Germination is really a double process, consisting in the breaking down of stored substances and in their resynthesis into material to be used for the newly developing parts of the young plant. In discussing the transformations of carbohydrates during germination, it has already been noted that the breaking down of starch and other polysaccharides into the simple sugars is followed by the synthesis of cellulose, which serves for the building of membranes of the new cells. The same is true of proteins. The reserve proteins are hydrolyzed, while the crystalline products of their disintegration, the amino acids, appear. These in turn give rise to ammonia. This stage, however, is followed by synthetic processes. Ammonia changes into asparagine, which, together with nonnitrogenous substances, forms new protein compounds, the living compounds of the protoplasm of the new cells.

The protein substances entering into the composition of the nucleus and the cytoplasm differ quite radically from the reserve proteins of the seed. Most of them belong to the complex proteins. The most studied of these are the nucleoproteins, which evidently form the main part of the chromatin of the nucleus. Nucleoproteins are very complex compounds. The pepsin of the gastric juice removes the protein group from these compounds, leaving the indigestible nuclein. By further hydrolysis, the nuclein can be broken down by splitting off another protein group and leaving only nucleic acid, which has nothing in common with proteins. Finally by still more thorough hydrolysis, the nucleic acid itself can be broken down into phosphoric acid, a carbohydrate group, and pyrimidine and purine bases.

Purine and pyrimidine are heterocyclic nitrogen-containing compounds. Their derivatives being substances of a basic character, they will combine readily with phosphoric acid. The carbohydrate group is most frequently represented by the pentose, *l*-ribose $C_5H_{10}O_5$. In breaking down nucleoproteins in the animal organism, the derivatives of purine most often give uric acid.

The proteins of the protoplasm have been studied very little. In protoplasm, glucoproteins are often found; these are complex proteins, which yield carbohydrate groups on hydrolysis. Fungi are especially rich in glucoproteins. Protoplasm contains also special phosphorus-containing proteins, which do not belong to the nucleoproteins and are called "plastins." Many authors, including Lepeschkin, suppose that the proteins of protoplasm, being of a very complex structure, are chemically bound still further with lipoids forming molecular complexes of an enormous size and capable of the most varied biochemical reactions. It was at one time supposed that "living" proteins of the plasma differ from "nonliving" ones in that nitrogen is found in them not in the form of the amino group, NH_2, but in the form of the cyanic group, —C:N, which gives them a special instability and high capacity for transformation—two essential properties of life. This theory was first advanced by Pflüger and was supported for a long time by Loew and Bokorny, who by means of a special aldehyde reaction attempted to distinguish chemically between living and nonliving proteins.

The most recent biochemical investigations do not support this idea, and therefore there is no reason to believe that the proteins of protoplasm are especially unstable. Quite the contrary; in their relation to enzymes and to many chemical reagents, they are usually more stable than the reserve proteins.

Such processes of hydrolysis of reserve proteins and synthesis of active proteins of the protoplasm also take place at the time of resumption of growth by other storage organs. Asparagine, for instance, accumulates in large quantities in the underground sprouts of asparagus, from which it has received its name. Considerable quantities of it are also to be found in the unfolding leaves and flower buds of trees that have been kept in darkness; but in the light, synthetic processes soon become dominant, proceeding at the expense of the newly produced carbohydrates.

A general picture of the metabolism of reserve substances is presented in the diagram in Fig. 16, borrowed from one of the papers by Prianishnikov. This diagram shows very clearly that the total amount of nitrogenous substances remains nearly constant even during a comparatively long germination period in the dark, in spite of the intense disintegration of proteins. The only change that takes place in this group of substances is the accumulation of amino acids and asparagine in exchange for

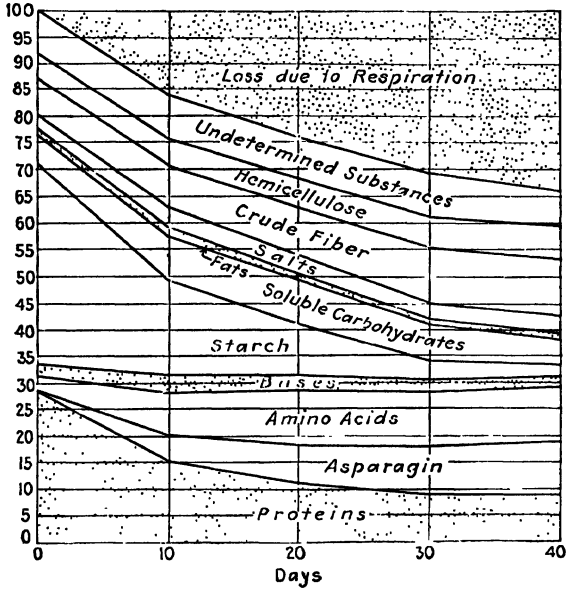


FIG. 16.—The transformation of reserve substances during the germination of vetch seeds (*diagram after Prianishnikov*).

the disintegrated proteins. Quite a different picture is presented by the carbohydrates. A rapid decomposition of starch takes place, but there is no accumulation of soluble sugar to correspond to the accumulation of amino acids; their quantity remains constant. The cause of this difference lies in the fact that the products of hydrolysis of starch are rapidly utilized in the process of respiration, which is very intense in germinating seeds. Almost the total decrease in dry weight of seeds that may be observed during the period of germination and that is represented in our diagram in the form of a shaded wedge at the top, corresponds to the amount of carbohydrates utilized in respira-

tion. In exchange, the seedlings have obtained free energy necessary for their growth and vital activity.

The general aspect of metabolism of reserve substances would not be complete without mentioning the metabolism of the mineral substances. An elementary analysis of seeds will show that besides the four essential elements participating in the formation of carbohydrates, fats, and proteins, *viz.*, carbon, hydrogen, oxygen, and nitrogen, they contain a number of other chemical elements. The most important of these are sulphur, phosphorus, potassium, magnesium, calcium, and iron. As has already been stated, some of these elements, *viz.*, sulphur and phosphorus, participate directly in the structure of proteins. Sulphur is present in all protein substances without exception, while phosphorus, or more exactly phosphoric acid, is an indispensable part of the proteins of the nucleus. Moreover, sulphur and phosphoric acid play a very important role in the process of respiration, which is very intense in germinating seeds. That is why there are always found in seeds reserves of compounds of sulphur and phosphorus, chiefly as part of the protein molecules. When these protein substances are stored in the form of aleurone grains, there may be observed under the microscope inclusions of phosphoric acid salts, as well as of other salts that have been generally termed "globoids." During germination, the globoids dissolve. The significance of other mineral elements during germination comes chiefly from the regulatory action of their ions upon the colloidal chemical processes taking place in the cell. The significance of cations has already been mentioned in the discussion of the question of the stability and coagulation of hydrosols. No considerable reserves of different cations are found in the seeds, however; for in natural conditions of germination in the moist soil, the seeds find and absorb sufficient quantities of necessary cations from the surrounding medium. But in germinating seeds in distilled water, one may frequently observe symptoms of acute deficiency in mineral cations. In such cases, the addition of necessary salts may be of great significance.

Since the question of the needs of seedlings for mineral salts is very difficult to separate from the problem of mineral nutrition of mature plants, a more detailed discussion of the subject will be deferred to Chap. VII, where the problem of the significance

of mineral substances in the life of the plant will be examined in detail.

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CHAPTER III

RESPIRATION OF PLANTS

19. Importance of Respiration in the Life of the Plant and the Nature of the Process.—Respiration is a process that has long been closely connected with life. Respiration is essentially the oxidation and decomposition of complex organic compounds, principally carbohydrates into simpler substances such as carbon dioxide and water. It may be expressed by the following general reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 674 \text{ cal.}$$

This reaction shows that oxygen absorbed from the air participates in this oxidation and that the chief product of this reaction is carbon dioxide gas liberated into the surrounding atmosphere in exchange for an equal volume of oxygen absorbed. This exchange of gaseous oxygen and carbon dioxide is the most prominent and most easily demonstrated external manifestation of respiration. Later on, it will be shown (Art. 21) that it is the quantitative changes in the surrounding atmosphere and not inside the living object that are used in most of the methods of studying the intensity of respiration.

The physiological significance of respiration is not determined merely by this external manifestation of a definite gas exchange. The process of respiration is the liberation of energy connected with the oxidation of organic substances. All the vital functions of the organism, such as growth and the movements connected with growth, and different kinds of reactions accompanying synthetic processes all require energy, just as it is required by motors in a factory. This energy is obtained from the oxidation of carbohydrates. However, unlike the burning of fuels in factory furnaces, in protoplasm the chemical energy of oxidation is directly transformed into other forms of chemical energy; and only after the physiological process has been completed, does it appear in the form of heat energy. These internal transforma-

tions of energy during respiration remain as yet insufficiently understood. The amount of energy liberated in the process of respiration is commonly determined by the heat liberated.

According to the laws of thermodynamics, the total amount of heat released in an exothermic chemical process does not depend upon the intermediate stages and transformations of the process. That is why it may be assumed that the total energy of the physiological process of oxidation (respiration) of carbohydrates and other organic compounds will be equal to the heat obtained in burning these substances in a calorimeter. For the combustion of glucose, the energy liberated as heat amounts to 674 kilogram-calories (Cal.) per gram molecule (180 g.). If the initial substances to be oxidized are not carbohydrates but proteins and fats, considerably larger magnitudes will be obtained. On the average, it may be assumed that in burning 1 g. of carbohydrates, 4 cal. are liberated; 1 g. of protein, 5.7 cal.; and 1 g. of fats, 9.2 cal. The greater liberation of heat by proteins and especially by fats as compared with carbohydrates is conditioned by their higher hydrogen and lower oxygen content.

Respiration is essentially of the same nature in both plant and animal organisms. It consists in obtaining energy for the cells during the combustion of carbohydrates. Usually, the animal organism consists of a massive body, to some parts of which gaseous oxygen cannot easily penetrate; hence, in order to provide all cells with oxygen, a special complex mechanism is required, which consists of a blood-circulating system with its conveyers of bound oxygen, the red blood corpuscles, and respiratory organs, where the blood can be saturated with oxygen and freed from the accumulated carbon dioxide. The complexity of these essential yet adventitious organs has obscured the true nature of respiration in animals. Even as late as the beginning of the nineteenth century, the opinion still prevailed that respiratory movements and blood circulation were the main features of respiration; and, consequently, the existence of respiration in plants was denied. Much effort was necessary to introduce into science the truth that respiration proceeds neither in the lungs nor in the blood, but in each living cell.

The external conditions of respiration are very much simpler in plants than in animals. Owing to the development of a

comparatively large surface, as a result of gaseous nutrition, the oxygen of the air comes in direct contact with each cell of the plant body. The oxidation processes therefore are not obscured by any accessory features. Naturally, then, the attention of botanists has been continually directed to the study of the nature of this phenomenon, and it is in plant physiology that the question of the internal chemistry of respiration has received special consideration.

The study of the process of respiration in the plant organism is connected with the names of the most eminent plant physiologists, such as Pfeffer in Germany, Bonnier in France, Blackman in England, and Borodin, Palladin, and Kostytchev in Russia. The attention directed to the study of respiration is explained by the fact that life in any organism is closely connected with a continuous expenditure of energy obtained from respiration. No wonder then that the study of the respiratory process represents one of the principal problems of general physiology and that a profound penetration into the chemistry and energy relations of this organic function promises a deeper penetration into the essentials of the specific complex of phenomena defined by the general term "life."

20. Methods of Studying Respiration. Intensity of Respiration of Various Plants and of Their Different Parts.—Study of the intensity of the process of respiration is performed by means of methods for determining the quantitative changes in the atmosphere surrounding the plants, *i.e.*, the accumulation of carbon dioxide and the decrease of the oxygen content.

The first investigations of the process of respiration by Saussure had the object of proving the existence of this function in plants and were performed in eudiometers, *i.e.*, in graduated tubes, sealed at the upper end and with the plants, more frequently germinating seeds, placed inside. By immersing the open end of such tubes in water, a definite volume of air is included in them (Fig. 17), which can be analyzed after a certain interval of time. This is accomplished by introducing into the tube first a solution of potassium hydroxide, which absorbs carbon dioxide, and after this a solution of pyrogallol in combination with alkali for absorbing oxygen. The decrease in volume of the gas after the introduction of each of these two reagents will show the amount of carbon dioxide liberated and that of

oxygen remaining after the experiment. The initial composition of the air being known, it is not difficult to compute the amount of oxygen absorbed during respiration.

To obtain a more complete picture of the gas exchange during respiration, at present it is found preferable to place the parts of the plants subjected to investigation in hermetically sealed containers, with tubes through which samples of air are taken from time to time by means of a special gas pipette. These samples are analyzed in a special apparatus permitting a very precise determination to 1 part in 10,000 on volumes of gas not exceeding 1 cc. Recently, McAllister at the Smithsonian Institution has used the spectrophotometer to measure the infrared absorption bands of carbon dioxide, which permits changes of 1 part per million to be quantitatively determined at 5-sec. intervals without removing an aliquot of the gas sample.

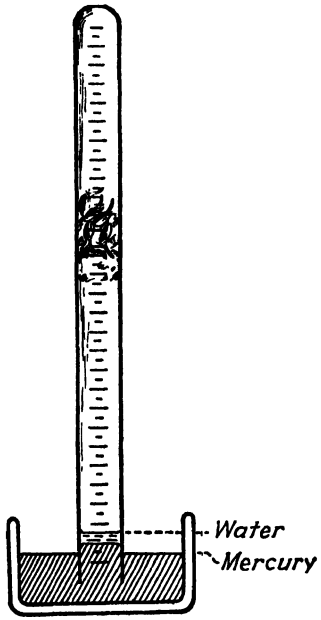


FIG. 17.—Study of respiration of germinating seeds by means of a eudiometer.

All experiments with green plants or those which are able to become green must, of course, be conducted in darkness, lest the opposite process of absorption of carbon dioxide should superimpose itself on respiration.

Often, the study of respiration is confined only to the determination of the carbon dioxide liberated, which of course, is more readily made than is a complete gas analysis. For this purpose, the respiring parts of a plant are placed in a container through which is drawn an air current that has been freed of carbon dioxide. Having passed over the plant, the air current leaves the carbon dioxide, caught on its way, in a special absorbing apparatus. This may be either a U-shaped tube containing potassium hydroxide or soda lime, or one of the various kinds of vessels containing potash. The quantity of carbon dioxide formed per unit of time is estimated by the increase in weight

of the apparatus. Previous to entering the apparatus, the current of air must be dried. Frequently, a titratable solution of barium hydroxide is used as an absorber of CO_2, with Pettenkoffer's tubes used as a convenient equipment (Fig. 18), through which an air current passes in the form of fine bubbles that discharge into the barium hydroxide their carbon dioxide. The quantity of the latter is determined by the difference between the initial and final titration figures of the solution.

The intensity of respiration may also be determined by measuring the amount of oxygen absorbed. To observe and measure the absorption of oxygen by the respirating organism, a small cup with caustic alkali is placed in the container holding

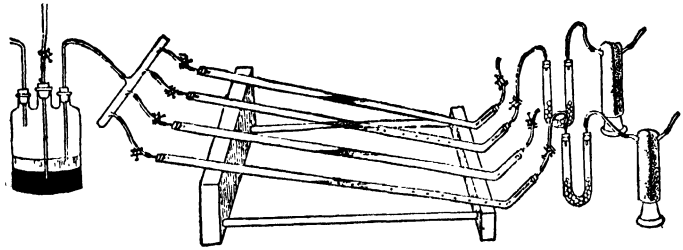


FIG. 18.—Pettenkoffer's apparatus for quantitative determination of carbon dioxide eliminated in respiration. The germinating seeds are placed in U-shaped tubes connected with cylinders containing soda lime and absorbing CO_2 of the air. The air current proceeds from right to left (*after Palladin*).

the respiring organs of the plant to absorb carbon dioxide liberated in the process of respiration. With such an equipment, the visible decrease of the total volume of gas in the container will correspond to the amount of oxygen absorbed during respiration. To measure the decrease in volume, the container is provided with a graduated lateral tube of small diameter forming a manometer. This apparatus is called a "respirometer" (Fig. 19). Not so long ago, this apparatus was assumed to be very crude and was used only for demonstrations to students of the phenomenon of absorption of oxygen. Recently, it has been considerably improved by Warburg, so that now it attains a high degree of precision and sensitivity. With an apparatus of this type Warburg performed important investigations on respiration, which will be further described in Art. 24.

There is considerable variation in the intensity of respiration in different plants and in different parts of the same plant.

Respiration is closely connected with growth; therefore, the greater the energy of growth the higher is the respiratory rate. Rapidly growing organs, whose cells are rich in protoplasm, often respire more intensely than do animals. Man, for instance,

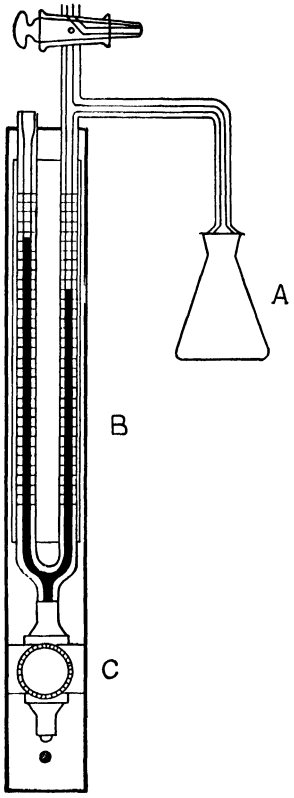


FIG. 19.—Warburg's apparatus for the study of respiration of plants. *A*, receptacle for the respiring parts; *B*, manometer pressure gauge; *C*, adjusting valve.

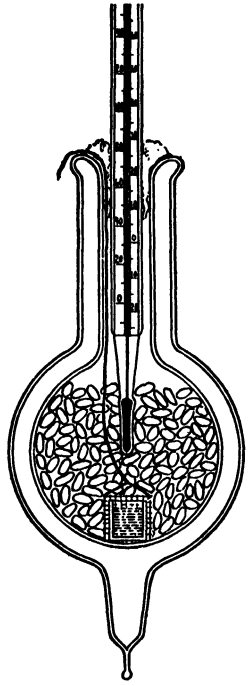


FIG. 20.—Flask for the study of increase in temperature induced by respiration of germinating seeds (*after Ganong*).

produces in 24 hr. a quantity of carbon dioxide weighing about 1.2 per cent of his body. The respiration of germinating seeds at a temperature of 37°C. is of the same intensity. Still greater is the respiration of molds, which produce as much as 6 or even 10 per cent of carbon dioxide in 24 hr. per unit weight.

The following table gives an idea of the quantity of carbon dioxide that is liberated by various plant organs during 24 hr. at room temperature per gram of dry weight.

Plant Material	Cubic Centimeters of CO_2 Released in 24 Hr.
Full-grown leaves	12 to 24
Growing rootlets	40 to 80
Buds of trees	35 to 70
Germinating seeds	60 to 120
Growing mycelia of fungi	270 to 1,800

With the maturing of plants, the intensity of respiration of its organs decreases sharply. Thus, for instance, Kidd, West, and Briggs (1921) observed that leaves of a sunflower plant 22 days old liberated 3 mg. of carbon dioxide per hour per gram of dry weight; 36 days old, 0.81 mg.; 50 days old, 0.46 mg.; 64 days old, 0.34 mg.; 99 days old 0.25 mg.; and 136 days old, 0.08 mg. Thus toward the end of the vegetative period, the intensity of respiration decreased to one-fortieth of the initial rate.

The object of respiration in plants is the liberation of energy for use in vital processes. On account of their enormous surface and a comparatively low rate of respiration, it is impossible for plants to maintain a temperature at a level much higher than that of the surrounding air. But when a considerable quantity of intensely respiring plant material, such as germinating seeds, flowers, or leaves, is put into an insulated container, *e.g.*, a Dewar flask (Fig. 20), a rise in temperature of 30 to 40 or even 50°C. may be observed. This extreme temperature may eventually result in death of the plant material. Still more accentuated is the rise of temperature during the respiration of fungi and bacteria. On damp hay, for example, there develops rapidly such a rich flora of microbes that it soon becomes very warm and may "heat" if it is not scattered. A considerable rise in temperature may be observed also in some large flowers. The flowers of *Victoria regia*, for instance, may have a temperature 12°C. higher than the surrounding air. In the inflorescence of *Arum*, *Colocasia*, and other aroids, this difference may be as high as 30°C.

Part of the energy liberated during respiration may take the form of light. Phosphorescence, however, is observed only in

the lower plants, the fungi and bacteria. Their activity explains the phosphorescence of rotted wood, sea fish, meat, etc. Phosphorescence may be due simply to oxidation by the air of special phosphorescing bodies of protein character produced by the organism.

According to the data of Gurvitch, root ends and some other parts of the plant where there is an intense multiplication of cells emit "mitogenetic rays," which are capable of stimulating the process of division in dormant cells. These rays are of a very short wave length (about 2500 Å.) and lie beyond the limits of the visible spectrum in the region of the ultraviolet rays. No definite proof, however, has been supplied of the existence of mitogenetic rays.

21. Substances Consumed during Respiration. The Respiratory Quotient.—The fundamental respiratory materials, as has been seen, are the sugars, especially fructose. From the general equation of respiration,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O,$$

it is clear that the volume of the gases exchanged, *viz.*, the oxygen absorbed and the carbon dioxide liberated, must be equal. A plant placed in a closed container, therefore, does not alter the volume of air, though it changes its composition, substituting carbon dioxide for oxygen. In the more typical cases, the ratio of carbon dioxide to oxygen in respiration, $CO_2:O_2$, called the "respiratory quotient," is equal to unity.

There are many deviations from this general rule, as very often the respiratory quotient is less than unity. Sometimes, it is more. Various causes may be responsible for these differences. Besides the end products of oxidation, CO_2 and H_2O, frequently there are formed but partly oxidized compounds—such as oxalic acid, $C_2O_4H_2$; tartaric acid, $C_4O_6H_6$; etc.—which contain more oxygen than carbohydrates do. When these substances are produced, part of the absorbed oxygen remains in the plant, and the ratio of $CO_2:O_2$ is less than unity. On the other hand, when oxygen is deficient, as in the respiration of bulky organs or seeds immersed in water, normal respiration may be accompanied by alcoholic fermentation, resulting in the liberation of carbon dioxide without absorption of oxygen from the air. In such cases, the respiratory quotient will be greater than unity.

The respiratory quotient may diverge considerably from unity in cases when the respired material is not sugar but some other substance that contains different quantities of oxygen and hydrogen from those that sugar contains. When the substance is richer in hydrogen, *e.g.*, a fat or a protein, part of the oxygen will be utilized in the oxidation not only of the carbon but also of hydrogen, and the respiratory quotient will fall as low as 0.7 to 0.8. In the oxidation of stearic acid, for instance, the reaction proceeds as follows:

$$C_{18}H_{36}O_2 + 26O_2 \rightarrow 18CO_2 + 18H_2O,$$

and the ratio of $CO_2:O_2$ will be 18:26 or 0.69.

In the combustion of substances containing more oxygen than do carbohydrates, such as the organic acids, the respiratory quotient, on the contrary, will be greater than unity. Thus in the combustion of oxalic acid, according to the equation

$$2C_2O_4H_2 + O_2 \rightarrow 4CO_2 + 2H_2O,$$

the respiratory quotient is 4. Hence, it is quite clear that the lower the respiratory quotient, the higher is the heat liberation during combustion, and vice versa. Proteins and fats exhibit therefore a high heat equivalent, organic acids a low one.

Variations in the heat equivalent depending on the food material supplied may be most readily observed in molds, which possess scarcely any reserve substances of their own but are endowed with a very high intensity of respiration. When such fungi are grown on carbohydrates, the $CO_2:O_2$ ratio is almost equal to 1; when cultivated on fats, it falls to 0.7; when on acids, it rises to 2 to 3. But if these organisms are supplied with a mixture of sugar and other substances, the coefficient will be at first close to 1 until the sugar is used up. Then the combustion of the other substances will begin with a consequent change in the ratio. It is interesting to note that even in oily seeds the quotient may be increased until it approaches 1, if sugar is supplied from without (Polocev, 1910). This shows clearly that sugar is the chief respiratory material. Kostytchev is of the opinion that sugar is the only directly combustible material, the other substances, such as proteins and fats, undergoing transformation with release of sugar, previous to combustion.

The predominant role of the sugars in nutrition, as well as in respiration, is, moreover, suggested by the interesting fact that molds develop more slowly on fats than on sugars. The reason of this is that fats must previously undergo a complex transformation, while sugars are utilized directly. The same is indicated by the transformation, described above (Art. 17), of fats into sugars during the germination of oily seeds.

22. The Dependence of the Respiratory Intensity on the Conditions of the Surrounding Medium.—Being one of the manifestations of the vital activity of protoplasm, respiration may be realized only within the limits of conditions of the surrounding medium that will permit the existence of living protoplasm. All factors killing protoplasm likewise inhibit the normal process of respiration. With some methods of killing the protoplasm that affect less profoundly the structure of the living substance—*e.g.*, killing either with chloroform vapor or other toxic substances or by freezing—after the death of the cells, the processes of oxidation and decomposition continue for some time, and are accompanied by a liberation of carbon dioxide and absorption of oxygen. These processes are of great theoretical interest, for their study allows one to penetrate more profoundly into the internal chemism of respiration. However, this is not the normal process of respiration, and thus it does not stand in contradiction to the general statement that respiration is inseparably connected with life.

Of the many factors affecting respiration, let us first examine the influence of temperature. Temperature is an environmental factor exerting an effect not only on all vital processes but likewise on those of a purely chemical nature. According to the rule of van't Hoff, the rate of a chemical reaction doubles or trebles with each increase of 10°C. in temperature. This acceleration of the reaction with an increase of 10°C. in temperature is designated as the temperature coefficient and is represented by the symbol Q_{10}. The van't Hoff coefficient may be expressed by the equation $Q_{10} = 2$. The temperature coefficient, however, is frequently more than two.

It must be emphasized that vital processes follow van't Hoff's rule only in comparatively limited temperature ranges, between 0 and 30 to 35°C. With a further increase of temperature, the acceleration slows down; then there follows rapid decrease in

velocity, the break of the curve displaying a sharp angle; and finally at a temperature of about 40 to 50°C., the process ceases completely. In the temperature curve of vital processes, there are found the three basic, or, as they have been termed by Sachs, cardinal points: the minimum, at which the process just starts; the optimum, when it proceeds with the highest velocity; and the maximum, in excess of which the process ceases.

The lower temperature limit of respiration of plants lies beneath −10°C. and is apparently conditioned chiefly by freezing of the tissues. In hibernating green parts of plants, *e.g.*, in the buds of deciduous trees and in needles of conifers, it is much lower. Perceptible respiration may be observed at temperatures about 20 to 25° below zero. With an increase in temperature, respiration increases rapidly and up to 40°C. follows the rule of van't Hoff, the coefficient Q_{10} varying from 1.9 to 3.9. Above 40°, there may frequently be observed a rapid jump upward, then at about 50° an abrupt fall, accompanied by the simultaneous death of the plant.

Investigations (Kujiper, 1910; Fernandez, 1921) show that essential corrections must be introduced in determining the optimum point, *i.e.*, the temperature of the greatest intensity of respiration. If the intensity of respiration of a plant placed for a prolonged period in definite temperature conditions is determined, it will be noticed that with temperatures in excess of 35°C., the process of respiration, initially very intense, soon begins to decrease and after some hours becomes considerably weaker than at temperatures about 25 to 20°C. and lower. This phenomenon may be explained by the fact that at temperatures in excess of 35°C., the protoplasm undergoes changes that very soon become manifest in a decrease of respiration. The temperature at which respiration first shows an increase and then is followed by a decrease must not be taken for the optimum. At the actual optimum temperature, the intensity of respiration remains continually at a high level. This optimum usually is between 35 and 40°C., at least for germinating seeds of common crop plants. It is interesting to note that this is the temperature that is found in man and in other warm-blooded animals and that is absolutely indispensable for the normal course of their vital processes.

Such a coincidence of optimum temperatures in so widely differing representatives of the organic world is certainly basically significant and serves as an indicator of the profound unity of the fundamental properties of their protoplasm.

Respiration is greatly influenced by the degree of swelling of the colloids of the protoplasm, increasing with the degree of their saturation with water. Dry seeds, containing only 10 to 12 per cent of hygroscopic water, do not display noticeable signs of vital activity. The well-known observations of Kolkwitz have shown that 1 kg. of dry seeds of barley liberated only 0.3 to 0.4 mg. of carbon dioxide per 24 hr. A small increase in the water content up to 14 or 15 per cent is sufficient to increase respiration 3 to 4 times, *i.e.*, up to 1.3 to 1.5 mg. of CO_2 per 24 hr. With further swelling, respiration increases very rapidly, and at 33 per cent water it reaches 2 g. per kilogram, *i.e.*, it increases over 10,000 times. With the beginning of germination, it increases still more. Similar data have been obtained for mosses and lichens. These plants on desiccation go into a state of inactivity but are capable of reviving after being moistened. A similar gradual slowing down of respiration may be observed in ripening seeds in connection with their desiccation.

The amount of material available for respiration in the cell also greatly affects the intensity of the process. As has been seen, this material is represented chiefly by sugars and other carbohydrates. The intensity of respiration is therefore rather closely connected with the presence and influx of carbohydrates and is considerably diminished in starving organs and cells. This explains the close connection between the respiration of leafy organs and their illumination, established by Borodin (1876). When kept in darkness, their respiration decreases gradually with the exhaustion of carbohydrates. After exposure to light and a renewal of the carbohydrate reserves, it increases rapidly. It has not been possible until recently to test the direct influence of light on the respiration of living cells. Recent experiments indicate that respiration is at the same rate, whether the plant is in light or in darkness, if other conditions are kept the same. An excessive illumination may cause an increase in temperature that will correspondingly affect respiration; on the other hand, it may affect the protoplasm injuriously, thus involving a decrease in respiration.

The mere presence of a considerable amount of material for respiration is not sufficient to provoke intensive respiration. In dormant storage organs, fruits, bulbs, and rhizomes, with reserve substances rich in sugars, the intensity of respiration is usually very low. Blackman, who has given much attention during recent years to the physiology of the storage of fruit, assumes that in the case of dormant plant parts a special inhibitory substance is present that does not permit respiration to proceed at a normal rate. He distinguishes two types of respirations. (1) The "flowing type" is determined by the presence of reserve substances of a carbohydrate nature and consists in their oxidation with liberation of carbon dioxide and water. (2) The second type of "protoplasmatic" respiration prevails in cases when reserve substances are exhausted and respiration is limited by the decomposition of substances of the active protoplasm itself. Such respiration serves to produce the minimum of free energy necessary for the maintenance of life.

A similar viewpoint had been previously advanced by Palladin, who also assumed that protoplasm is the basic factor of respiration. Proceeding from the fact that the proteins of the protoplasm and the nucleus (plastin, nucleoproteins) are digested by pepsin with great difficulty, he made an attempt to compare the intensity of respiration of the different organs of plants with their content of proteins that are not digested by pepsin. In such a simplified form, Palladin's hypothesis proved to be untenable, and he was forced to reject this method of determining the amount of active proteins. However, his original statement that it is essential to distinguish the effect of the oxidizing mechanism from the effects of oxidizable substances proved to be correct and formed the basis of contemporary theories of respiration.

Very abrupt changes in the intensity of respiration may be observed under the influence of various toxic substances. In small doses, nearly all such substances prove to be stimulating; *i.e.*, they increase the amount of carbon dioxide liberated. In large doses, however, they are toxic to plants, and respiration decreases rapidly. Special attention has been given to the study of such anesthetics as ether, ethylene, and chloroform. The same stimulative effect is displayed by a series of harmless substances, such as neutral salts of alkalies and alkaline earth metals, when applied in considerable concentrations. Such

effects should be considered as irritations and are characterized by an increase of respiration of a temporary nature that after a short period of increased rate returns to the initial intensity.

Not only chemical substances but also physical agents may act to irritate or stimulate the cell. For instance, abrupt changes in temperature, whether an increase or a decrease, may cause a temporary speeding up of the process of respiration. The same effect may be produced by a change of light intensity. A sharp and prolonged stimulation is caused by mechanical injuries to plant tissues, especially such as occur in the cutting of massive organs into pieces. In this case, there is the additional effect of the improvement of the conditions of gas exchange caused by the increase of the surface of contact with the atmosphere. Various deeply penetrating radiations, such as X rays, rays from radioactive substances, as well as ionization of the air and other electric influences, also stimulate respiration.

In some cases, there may be observed the depressing effect of external agents upon respiration. Extremely interesting and of great practical importance is the influence of carbon dioxide. When accumulated in considerable amounts, it decreases or inhibits respiration, as well as the processes of growth closely connected with respiration. Such an accumulation of carbon dioxide may frequently be observed in seeds with hard seed coats. This is probably the reason why they do not show signs of germination when moistened until the seed coat is mechanically injured. This explains the striking fact that seeds of many weeds and other plants may remain for years in the moist soil, without germinating and without losing their germination capacity. Their embryos remain in a state that may be compared to a condition of profound anesthesia under the influence of some anesthetic. Carbon dioxide gas may be considered as belonging to the group of anesthetic substances.

Recently this action of carbon dioxide is being utilized for the preservation of fruits and vegetables. Placing them in an atmosphere containing carbon dioxide at regulated concentration in a closed container inhibits the growth of certain pathogenic fungi and bacteria that otherwise would attack the fruit. Likewise, this procedure inhibits to a considerable degree the processes of senility and decomposition of the fruit itself. Thus their storage life is considerably increased.

Within a fairly wide range, the amount of oxygen in the air has no perceptible influence on the intensity of respiration. Only when the amount of oxygen has decreased to about 8 per cent does the respiratory quotient begin to increase perceptibly. Alcohol then accumulates in the tissues of the plants as a result of anaerobic respiration, *i.e.*, respiration without oxygen, which replaces normal respiration in conditions of a deficiency of oxygen.

23. Anaerobic Respiration and Alcoholic Fermentation.—Normal respiration represents an oxidation process that continues during the uninterrupted absorption of oxygen of the air. When a plant is transferred into an oxygen-free atmosphere, respiration must inevitably cease. Together with it are discontinued the processes directly connected with the liberation of energy obtained through respiration, *e.g.*, growth and protoplasmic as well as organic movements. When the plant is permanently deprived of oxygen, it will perish, since for its support, life requires a continuous supply of energy.

In the absence of free oxygen, death of a plant does not occur immediately, since life may be supported in the cells for some time by anaerobic respiration. As, in both normal and anaerobic respiration, carbon dioxide is eliminated, oxidation processes take place in both cases. In the absence of air, however, the necessary oxygen is taken from various compounds, *e.g.*, from water or the hydroxyl groups of sugar molecules; hence, oxidation of one organic substance or group during anaerobic respiration is accompanied by reduction of another organic substance or group.

Carbon dioxide is the most oxidized product of anaerobic respiration, while alcohol is a highly reduced product. The general reaction may be represented by the following equation:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH + 25 \text{ cal.}$$

When compared with the amount of free energy released by complete oxidation of a molecule of glucose, 674 cal., this quantity is very small. It is but natural, therefore, that the energy of anaerobic respiration is not sufficient to maintain all the life functions that usually are supported through ordinary respiration.

Besides the low energy value, anaerobic respiration has the other disadvantage of accumulating alcohol, a substance known

to possess toxic properties. Consequently, in the absence of oxygen, a plant perishes not merely from an excessively low supply of energy but likewise from self-poisoning. In this respect, the experiments by Nabokich (1905) are very instructive. He placed germinating pea and sunflower seeds in a large vessel containing water and by continued evacuation removed the last traces of air. The seeds not only remained alive for several weeks but even showed some growth. This is explained by the fact that the toxic products of anaerobic respiration were leached out by the water. In a gaseous oxygen-free medium, the same seeds exhibit no signs of growth. They die after 3 to 5 days.

The usual material for anaerobic as well as normal respiration is sugar. Other substances, *e.g.*, glycerin, mannite, tartaric and quinic acids, or peptone, may also be utilized. It appears, however, that the breaking down of these substances is preceded by their conversion into sugar, or at least into closely related substances.

Anaerobic respiration, which in the higher plants is but a temporary substitute for normal respiration, is in certain microorganisms the fundamental process of securing necessary energy. Yeast is a most conspicuous example of such microorganisms. Its anaerobic respiration is called "alcoholic fermentation."

Alcoholic fermentation has been known to mankind since time immemorial. It is used not only in the preparation of beverages, where the alcohol is produced by yeast, but also in breadmaking, where the counterpart of fermentation, the elimination of carbon dioxide, is used to raise the dough. For a long time, the nature of this process remained unknown. It was often ascribed to a purely chemical "autodecomposition" of sugar. By his classical investigations, Pasteur (1860) succeeded in ascertaining correctly the biological nature of alcoholic fermentation and in proving that fermentation is due to the vital activities of yeast cells and that it is indispensable to their existence in an oxygen-deficient medium. The significance of fermentation has been briefly formulated by Pasteur in the following statement: Fermentation is life without oxygen ("oxygen" meaning, of course, oxygen from the air).

For a long time, fermentation was thought to be the result of the general types of metabolism of substances in the yeast cells. It was assumed that yeast first assimilates sugar and then, after a

series of transformations of the sugar molecule, excretes alcohol and carbon dioxide as waste products. A series of investigations proved, however, that fermentation and the general vital activity of yeast are not identical. If the yeast cells are given pure sugar without nitrogen and mineral substances, they will cause fermentation but the cells will not multiply. On the other hand, in the presence of free oxygen, fermentation will be slightly retarded, but the reproduction of yeast cells is favored by the presence of the free oxygen. Therefore, for obtaining alcohol, it is more advantageous to limit the access of oxygen; while for purposes of increasing the quantity of yeast, *e.g.*, in yeast factories, it is more advantageous to give abundant aeration of the liquid medium.

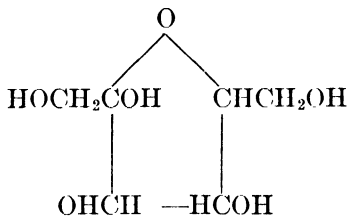
The most convincing evidence that fermentation may be separated from other manifestations of the vital activity of yeast cells has been given in the experiments of Buchner. In grinding yeast with sand and subjecting the mass obtained to a pressure of 300 to 400 atmospheres in a hydraulic press, he succeeded in securing a transparent juice not containing any living cells that nevertheless possessed the capacity of fermenting sugars. The active part of this juice could be precipitated by either acetone or alcohol and then again dissolved without loss of its capacity of producing fermentation. For this reason, it was considered an enzyme and was termed "zymase." Later, simpler methods of obtaining zymase were elaborated, *e.g.*, by means of treating the yeast cells with acetone or extraction with water after careful drying. This greatly facilitated the study of the enzyme. Further investigations proved that zymase represents not a single enzyme but a mixture of enzymes; and accordingly the reaction of alcoholic fermentation proves to be very complicated, proceeding in several separate phases.

These phases have not yet been definitely established, though the recent investigations of Neubauer and Kostytchev and their coworkers have brought us close to the solution of this problem. According to these investigations, the chief intermediate products of fermentation are pyruvic acid, $CH_3COCOOH$, and acetaldehyde, CH_3CHO.

Under normal conditions, these intermediate products do not accumulate in the fermenting liquid; but by introducing various substances, it is possible to change the course of the reaction

and cause an abundant formation of one of these products. By the introduction of sodium bisulphite which combines with acetaldehyde, large quantities of glycerin may be accumulated. This method is being widely applied in industry for the production of glycerin by the use of carbohydrates as a source instead of fats.

A fact of great interest is the participation of phosphoric acid in the reactions of fermentation. It has been proved that phosphates greatly accelerate the fermentation of sugars, sometimes as much as ten to twenty times, especially in the case of fermentation being produced not by living yeast but by the enzyme zymase. Phosphoric acid therefore is designated by many authors as the coenzyme of alcoholic fermentation. The investigations of Ivanóv (1905) and Harden and Young (1905) have shown that in the first stages of fermentation a diphosphoric ester of fructose, $C_6H_{11}O_4(Na_2PO_4)_2$, is formed, the so-called "zymophosphate," this synthesis being assisted by a special enzyme phosphatase. With the further course of fermentation, the hexose diphosphate disintegrates under the influence of the hydrolytic enzyme, phosphatase always producing fructose regardless of the kind of hexose from which it was formed originally. The cause of such an accelerating effect of phosphatase on fermentation is not entirely clear. It is possible that the transformation of all kinds of hexoses into fructose may be somehow connected with the existence of a special unstable modification, the so-called "gamma" (γ) fructose, which according to recent data is the most active sugar, participating in all vital processes. It is postulated as having the following structure:



Other sugars, hexoses as well as pentoses, give a similar γ form, and it is in this unstable form that they react most easily. It is interesting to note that in the molecule of sucrose, fructose is present in an active form and in the case of inversion

we first obtain γ-fructose, which later transforms into its inactive modification. It is very probable that this capacity of liberating the most active of all hexoses explains the presence and significance of sucrose in plant and animal organisms.

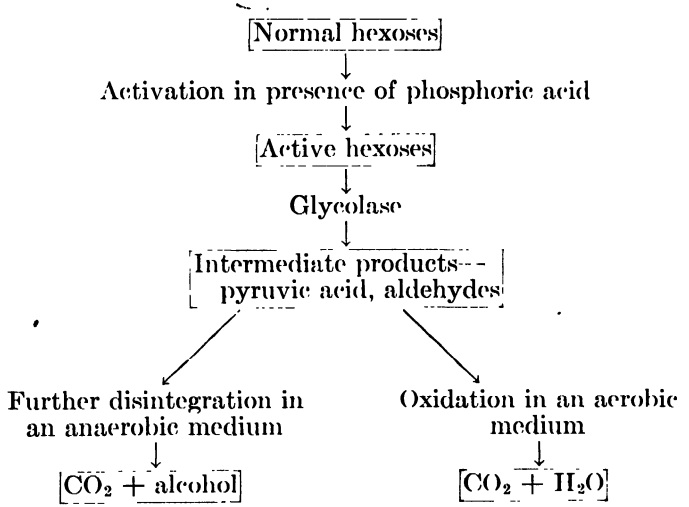
All these investigations show that both alcoholic fermentation and anaerobic respiration, which are closely allied in their chemism and according to the opinion of some authors are identical, represent a complicated catalytic process brought about not only by one, but by several enzymes. Some of them, *e.g.*, glycerophosphatase, belong to the group of hydrolases, *i.e.*, enzymes catalyzing hydrolysis, and the reverse synthesis. Others, *e.g.*, the glycolytic enzyme, which causes the disintegration of monoses into shorter chains, pyruvic acid or glyceric aldehyde, as well as carboxylase, which splits CO_2 from pyruvic acid, belong to the group of desmolases, for they cause a disintegration of the carbon chain. At present, the term "zymase" is used for designating this whole group of enzymes, the activity of which ultimately results in the disintegration of sugars into alcohol and carbon dioxide. In living cells, all these enzymes work in strict coordination, and fermentation proceeds as smoothly as if it were a simple reaction. But in a killed cell or in the expressed sap, the system is disturbed, and fermentation soon ceases. Hence the total quantity of sugar fermented by various preparations of zymase is always insignificant if compared with the amount fermented by living yeast cells. How exactly this coordinating action of the living protoplasm is realized is not known.

24. Relation between Fermentation and Respiration. Theories of Respiration of Palladin and Warburg.—It has already been pointed out that higher organisms, when transferred into an oxygen-free air, display anaerobic respiration, which in its main features is essentially the same as alcoholic fermentation caused by yeast. Therefore, the view has been established that alcoholic fermentation and respiration are closely related phenomena, the former being but a preparatory stage for the latter.

Formerly, the connection between fermentation and respiration was supposed to be of the following nature: The first products formed from sugar are alcohol and carbon dioxide; in an oxygen-free medium the process stops at this point, but in the presence of air, the alcohol obtained is oxidized further to carbon dioxide

and water. This supposition has been abandoned because during respiration alcohol is oxidized less readily than sugar. The process is now commonly supposed to take place in the following way: Under the influence of zymase, which is always present in cells, some intermediate products of alcoholic fermentation are formed first; the subsequent fate of these products is varied. Alcohol and carbon dioxide are formed from them when oxygen is lacking. In the presence of oxygen, they break down to carbon dioxide and water before alcohol has been formed.

The interrelations between anaerobic and normal respiration in the presence of oxygen may be represented in the following scheme, according to Blackman:



According to this scheme, based principally on the works of Palladin and Kostytchev and their coworkers, respiration must be viewed as the sum of fermentative processes, which may be divided into three phases: (1) The activation of normal inactive sugars and their transformation into an active form, realized in the presence of phosphoric acid and the enzyme phosphatase; (2) glycolysis, *i.e.*, the disintegration of active hexoses with the formation of very unstable intermediate products of the type of pyruvic acid, glyceric aldehyde, etc., brought about by enzymes of a desmolytic character; and (3) oxidation of these products by oxygen of the surrounding medium through oxidation enzymes.

The development of thought on the chemical nature of respiration proceeded for a very long time in two directions, which seemed to be irreconcilable. One theory was based on the activation of hydrogen, and the other, on the activation of oxygen.

The theory of activation of hydrogen was developed chiefly by Wieland. According to Wieland's theory, during the processes of oxidation that take place in complex systems containing the atoms of carbon, hydrogen, and oxygen in different combinations, one phase of the system of oxidized substances loses hydrogen, while the other gains it. The substances binding hydrogen are called "hydrogen acceptors," and the first phase of oxidation may be expressed by the following equation:

$$xH_2 + \text{acceptor} \rightarrow x + \text{acceptor } H_2.$$

In the following and final stage of oxidation, the hydrogen, united to the acceptor, connects with oxygen. The acceptor, being freed, may again take on hydrogen from the substance oxidized.

Wieland extended his theory of dehydrogenation to processes of oxidation-reduction that proceed with the introduction of the elements of water. Thus for instance, according to his opinion, in oxidation of aldehydes a hydrate is first formed through addition of a molecule of water.

$$CH_3COH + H_2O \rightarrow CH_3CH(OH)_2.$$

Then 2 atoms of hydrogen are transferred to the acceptor.

$$CH_3CH(OH)_2 + \text{acceptor} \rightarrow CH_3COOH + \text{acceptor } H_2.$$

The role of the acceptor is frequently carried out by a second molecule of aldehyde, which transforms into a molecule of alcohol. Thus one of the most important reactions of oxidation-reduction, taking place readily in water solutions of aldehydes and leading to a simultaneous formation of alcohol and acid, is the Cannizzaro reaction. It is usually represented by the following simplified equation:

$$\begin{array}{rl} CH_3COH & H_2 \rightarrow CH_3CH_2OH \\ + & | \\ CH_3COH & O \rightarrow CH_3COOH \end{array}$$

The Cannizzaro reaction was very often used to explain the processes taking place during fermentation (Neuberg, Kosty-

tchev, Embden, Meyerhof, and others) and is one of the most important oxidation-reduction processes occurring in the living organism.

According to Wieland, it is the mechanism for splitting off hydrogen that is catalyzed, and he terms this "catalyst activation." The removal of oxygen from the system in the form of water is the result of this activation; for the activated hydrogen is capable of reducing even molecular oxygen, hydrogen peroxide, H_2O_2, being formed first and then water. The accumulation of toxic peroxide in the organism of plants is inhibited by the enzyme catalase always present in the cell. It decomposes peroxide with the liberation of free oxygen. In the process of respiration, this oxygen is again combined by the activated hydrogen.

According to Wieland's theory, the chief catalysts of respiration are dehydrogenases, enzymes liberating hydrogen. The most important among them are the aldehydrogenases, catalytically accelerating the destruction of aldehydes and playing an important role in the process of fermentation; the peroxidases, contributing to the oxidation of polyphenols, the respiratory pigments of Palladin; and the hydrogen acceptors, participating in the oxidation of intermediate products of fermentation.

The chemist Schönbein (1860) was the first to formulate the idea of the activation of oxygen. He noticed that many chemical substances that by themselves do not oxidize in the air begin to oxidize in the presence of some autooxidizable substances. To explain this fact, Schönbein made the suggestion that oxygen is first combined by these easily oxidizable substances and thereupon is transformed into an easily activated form.

Proceeding on the basis of these views, which were further elaborated by Traube and Engler, Bach constructed his theory of the action of oxidizing enzymes, or oxidases. This idea includes the concept of organic peroxides, compounds of the general formula:



The oxygen of these substances may be partly or wholly liberated in the form of separate atoms with unsaturated affinities and consequently capable of oxidizing substances that are

not oxidized by the molecular oxygen of the air. According to Bach, these substances are oxygen acceptors and have been termed "oxygenases." As such, they possess little capacity for oxidizing compounds that are difficult to oxidize; they must first be subjected to the action of certain enzymes, "peroxidases," that split off an atom of oxygen.

The theory of Bach served as a basis for the theory of respiration suggested by Palladin. The latter author assumed that the primary acceptors of oxygen were "respiratory chromogens," substances of a phenolic nature, readily oxidized in the air and giving "respiratory pigments" of a quinone-like structure and easily demonstrated in plant juices owing to their dark color after oxidation.

The theory of Bach and Palladin created great interest but could not entirely explain the chemistry of respiration; for it gave a satisfactory explanation for the oxidation only of polyphenols, and attempts to connect this oxidation with the oxydative decomposition of sugars remained of a purely theoretical nature. The recent investigations of Warburg represent a very important advance in the study of the process of respiration. He made an attempt to apply the well-known oxidizing power of iron salts, always present in the living cell, to explain the process of respiration.

Warburg interprets the activation of oxygen by the fact that iron, one of the components of complex organic substances, combines with oxygen from the atmosphere and then transmits it to the oxidizable substance. In this process, iron in the ferric condition (Fe^{+++}) is reduced to the ferrous condition (Fe^{++}), which again may combine with oxygen. Proof of the essential significance of iron in respiration Warburg finds in the fact that hydrocyanic acid and hydrogen sulphide are extremely toxic cell poisons that stop respiration. According to his opinion, these poisons block the active atoms of iron, by forming stable compounds with them, and thus they lose their capacity of combining with oxygen. Warburg observed this phenomenon by means of a model of respiration that he constructed using carbonized hemin. This model possessed the ability to oxidize amino acids, in particular cystine. These experiments brought him to an understanding of the primary significance of iron in the process of respiration.

Further investigations of Warburg have shown that cell respiration is conditioned by a very definite iron-containing system, which he designated the "respiratory enzyme." This enzyme is a substance of the group of hemins, complex combinations of iron with a pigment, porphyrin, that contains four pyrrol nuclei.

Hemins enter into the composition of other cell substances, in particular the red pigment of blood, hemoglobin, but the respiratory hemin, or hemin enzyme, of Warburg differs in its structure as well as in its greater activity with respect to oxygen. It forms only a negligible part, less than 0.5 per cent, of all the pyrrol compounds of the cell. As it is the primary catalyst of oxidation, Holden calls it "oxygenase," *i.e.*, by the same name that Bach gave to the hypothetical enzyme that leads to the formation of peroxides.

The theory of respiration elaborated by Warburg, based on the activation of oxygen by the hemin enzyme, was at first thought to be in opposition to Wieland's theory, based on the activation of hydrogen. But in recent years, a series of attempts have been made (Oppenheimer and others) to unite both theories in a coordinated scheme of respiration, based on the assumption that in the process of respiration there is the activation of both oxygen and hydrogen and that activated hydrogen reacts only with activated oxygen. It is not possible to examine the details of all these theories, which at present are in a state of continuous change and elaboration. During recent years, Warburg has discontinued the assumption that the hemin enzyme is the sole respiratory enzyme and has discovered in yeast an unstable substance of an orange color that he has named the "second respiratory enzyme." This yellow enzyme does not contain any iron and is not hemin.

25. Energy Relations of Respiration. Oxidation-reduction Potential.—Respiration represents the main process by means of which the cell and the whole plant obtain energy, which is used for a series of vital processes such as movement and growth. At the time Lavoisier conceived the chemical nature of combustion, respiration was very often termed "physiological combustion," and the substances oxidized in the process of respiration were compared to the consumption of fuel by the furnace of a power plant, thus converting the chemical energy liberated by

combustion into mechanical energy of motion. This comparison however is not correct, since the living cell does not act like a heat engine, and the heat it liberates is not an intermediate but a final stage of the intracellular transformations of energy.

The transformations of energy connected with respiration in the living cell are far from being understood in all their details, just as the chemical processes involved in respiration are not completely clear. A considerable advance in the analysis of this question has been made through studies of oxidation-reduction potentials.

Oxidation for a long time was considered as being the addition of oxygen to the oxidizable molecule, and reduction as being the removal of oxygen. As has already been seen, further study of the reactions involved have led to considerable modifications of this idea. The dye, methylene blue, for instance, may be easily obtained by means of oxidation of its chromogen, a colorless leuco compound, but this oxidation consists not in the addition of oxygen to the leuco compound, but in the removal of two of its hydrogen atoms, which form with oxygen a molecule of water. Since it has been proved that such an oxidation with a removal of hydrogen is very widespread, all losses of hydrogen should be considered reactions involving oxidation.

Further expansions of the concept of oxidation went so far that this expression was applied not only to the transformation of ferrous oxide into ferric oxide by the addition of oxygen, but also to any transformation of ferrous into ferric forms, *e.g.*, the transformation of $FeCl_2$ to $FeCl_3$. And as this transformation is based on the change of valence of iron, the transformation of the ferrous ion Fe^{++} into a ferric ion Fe^{+++}, the term "oxidation" was applied to any acquisition of a positive charge by an ion; from the viewpoint of the electron theory, this will be the case of a loss of one or more electrons. According to this terminology, reduction is the acquisition by an atom of one or more electrons.

Thus the phenomenon of oxidation and reduction can be combined with the phenomenon of gaining or losing electrons. This concept made possible quantitative determinations of the energy relations of these phenomena, based on the accompanying changes in the electric charge of a system, where these processes are taking place. If a platinum electrode is placed in an aqueous solution of two salts of one and the same metal but of a different

degree of oxidation, *e.g.*, $FeCl_2$ and $FeCl_3$, the electrode will assume a potential dependent upon the proportional concentrations of the two ions Fe^{++} and Fe^{+++}. If Fe^{++} ions are in excess of Fe^{+++} ions, the electrode will be negatively charged. On the other hand, with prevalence of Fe^{+++} over Fe^{++} the electrode will be charged positively. If the two solutions are placed in separate containers, divided by a porous membrane and with platinum electrodes in each, and the electrodes are connected to a galvanometer, a current will flow owing to the different potentials assumed by the two electrodes. In the containers, there will take place, in one Fe^{+++}, reduction to Fe^{++} ions; and, in the other Fe^{++}, oxidation to Fe^{+++}: and these processes will stop only when there is an equal proportion between the ions Fe^{+++} and Fe^{++} in both solutions. Thus under conditions permitting the exchange of charges, there is oxidation or reduction of ions carrying different charges, and there is obtained an oxidation-reduction system, which may produce energy, depending upon the difference in the potentials of the two immersed electrodes.

The electrical potential that is assumed by a platinum electrode when immersed in an oxidation-reduction system represents a definite magnitude for each solution. The magnitude of this potential, called the "reduction oxidation" or "redox" potential, indicates the direction in which the reaction will proceed. Substances having a higher potential will oxidize substances having a lower redox potential, and conversely the latter will reduce the former. The highest potential found in plants may be taken as that of an electrode in pure oxygen; the lowest, that of an electrode in pure hydrogen. Most of the oxidation-reduction systems in plants possess an intermediate potential; and according to their redox potentials, plant substances may be placed in a series in which each preceding member may oxidize each following member, and conversely the latter may reduce the former.

The determination of the redox potential may be accomplished by means of the electrometric method, just like the determination of pH. Various organic dyes also are used as indicators for this purpose. The use of redox indicators is based on the fact that most of them are transformed with reduction into colorless leuco compounds or have different colors at different redox potentials.

The value of the redox potential can be determined. It is a function of the hydrogen-ion concentration.

A very important feature of oxidation-reduction systems is the reversibility of the reaction, which permits the maximum utilization of the energy developed without its degradation to heat. The presence of reversible oxidation-reduction systems in the plant cells is of great significance, because through this mechanism the chemical energy of substances can be transferred to other molecules without passing into heat, as would occur if respiration, for instance, were merely a combustion of the substance. The fact has already been emphasized that the existence of oxidation-reduction systems is assumed in most of the theories of respiration. Thus Palladin stresses the significance of respiratory pigments, which are continually oxidized and reduced and which transfer oxygen to intermediate products of fermentation or remove hydrogen from them. In Warburg's theory, great importance is ascribed to iron with transformation from the ferrous Fe^{++} to the ferric form Fe^{+++}, and the reverse. Recently, in connection with the investigations of Hopkins (1929, 1935), a great significance is attributed to organic compounds of sulphur containing the sulphydryl group —SH. With oxidation, which is actually the subtraction of hydrogen, 2 molecules of such compounds combine by means of their sulphur atoms into a double molecule, and the hydrogen released serves for reduction. Of such sulphur-containing compounds, the greatest significance is ascribed to the amino acid cysteine, $SH{\cdot}CH_2{\cdot}CHNH_2{\cdot}COOH$. Schematically it may be represented by the formula RSH. Its oxidation and reduction may be represented by the following equation:

$$\underset{\text{Cysteine}}{2RSH} \leftrightarrows \underset{\text{Cystine}}{RSSR} + 2H.$$

Two molecules of cysteine are combined to 1 molecule of cystine; as has been seen in Art. 12, these are among the hydrolytic products of proteins.

In animal and plant cells, it is not free cysteine but its combination with glutamic acid, glutathione, that is of major importance. Glutathione is a dipeptide and may be represented by the following formula:

$$\begin{array}{l} CH_2{\cdot}SH \\ | \\ CH{\cdot}NH{\cdot}CO{\cdot}CH_2CH_2CH{\cdot}NH_2COOH \\ | \\ COOH. \end{array}$$

Alkaline reactions favor the oxidation of glutathione, while the reduction of the oxidized form proceeds more readily in an acid medium. Like all compounds containing the group SH, glutathione possesses a very low redox potential; *i.e.*, it is a strong reducing agent.

The reverse may be said of systems containing ferric iron. They possess high redox potentials; *i.e.*, they are strong oxidizing agents. Neither ferrous nor ferric ions are found in cells in a free state, but iron is always found in the form of organic compounds *i.e.*, "bound" or "masked" iron. Among such compound may be mentioned Warburg's respiratory enzyme, hemin. In the animal organism, hemoglobin containing iron plays an important part as a carrier of oxygen. Closely allied to hemoglobin is the pigment, cytochrome, discovered by Keilin (1925), which is very widespread in plants, but whose significance is disputed by many authors. In each cell, therefore, there are highly positive and highly negative redox systems, the interrelations of which determine the course of the process of respiration. But an understanding of all the details of these interrelations forms the problem of future investigations.

It is important to note one essential difference of the new viewpoint on respiration. While previously respiration was identified with combustion, the new viewpoint considers respiration a complex system of oxidation-reduction processes. Combustion represents a typically irreversible process, all the energy of which is released in the form of heat. The identity of respiration with combustion would make it quite impossible to understand how heat energy might be directed to the accomplishment of other vital processes, such as growth and synthetic processes. Considering respiration as being a complex combination of oxidation-reduction processes, it may easily be imagined that the electrochemical energy liberated in these processes is used in other chemical reactions that require energy. Only after the energy quanta have been transformed into small quanta repre-

sented as heat wave lengths do they appear as heat that has no direct significance for the plant.

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CHAPTER IV

GROWTH OF PLANTS

26. Growth of Plants. Germination of Seeds.—One of the most important manifestations of the vital activity of plants is their growth, their continuous increase in size.

Growth is closely connected with the formation of new cells, tissues, and organs of plants as well as with the increase in volume of the cells. Any reversible increases in volume, resulting, for instance, from the swelling of seeds in water, are not considered as growth. Growth is usually combined with a corresponding increase in the total mass of the plant, but there are cases where growth is accompanied by an expenditure of substance. For instance, during the first stages of germination of seeds or the utilization of reserves from storage organs when the leaves have not developed, there may be observed considerable losses of organic substances due to respiration, but new cell formation occurs.

In such cases, the total increase in the size of the seedlings takes place on account of the water absorbed during new cell formation with the translocation and use of substances for new cell structures. Swelling that predominated in the first stages of germination is later supplanted by the increased water-holding capacity of structures formed during the growth of new cells. On drying germinated seedlings, it will be observed that there is a decrease, not an increase, in dry substance, which may amount on prolonged germination to over 50 per cent of the initial weight of the seeds. In this case, the growth of seedlings is combined not with an accumulation but with a redistribution of organic substances. Organic substances that have been stored during ripening of the seed are partly used for the creation of protoplasm as well as for laying down the walls of the newly formed cells; they are consumed, also, to obtain the energy necessary for growth.

These two opposite functions, the consumption of reserves and the creation of new tissues, which characterize germination and

growth are localized in different regions of most seeds. From a physiological point of view, there may be distinguished three main parts of the seeds: (1) the seed coats covering the seed from the outside and serving as a protection from various injuries and unfavorable external conditions; (2) the embryo, consisting of a rudimentary rootlet, leaves, and a stem uniting them; and (3) reserve tissues, occupying usually the greatest part of the volume of the exalbuminous seed, when the reserves are stored mainly outside of the embryo itself in the endosperm. The endosperm may be vanishingly small in seeds whose reserves are stored in the cotyledons (albuminous seeds).

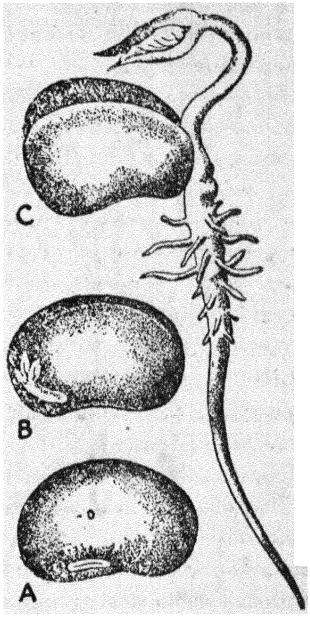


FIG. 21.—Seed of beans: A—externally; B—from the internal side of the cotyledon; C—growth of the embryo parts of the seed.

Storage in the cotyledons can be observed in seeds of peas, beans, and in most dicotyledonous plants (Fig. 21). Storage in the endosperm can be observed in seeds of cereals and grasses. The endosperm is of an origin quite different from that of the embryo. It originates not from the fertilized egg but from the union of primary endosperm nuclei, and subsequent cell divisions after fusion with the second generative nucleus of the pollen tube. The endosperm nucleus is thus formed by the fusion of three nuclei and has $3n$ number of chromosomes. The endosperm represents a uniform parenchymatous tissue almost entirely filling the seed of cereals, the embryo being pushed to one end. This structure may be observed in the seeds of most monocots (Fig. 22). Besides these two main types of seed, there exist also others of less importance.

The germination of seeds begins with swelling. With the development of the embryo, the seed coat bursts, ceasing to impede further growth. The growth of embryonic parts of seeds proceeds at the expense of the reserve substances, which are decomposed by enzymes in the processes of hydrolysis. The

depletion of the reserve organs of the seed is usually accompanied by their shriveling and desiccation and finally their detachment from the plantlet after the embryo has attained complete independence. With the endosperm, this is always the case. Frequently it is entirely dissolved and absorbed by the seed, so that only the empty seed coat remains. When the reserve organs are cotyledons and are carried above ground, they usually become green and begin to function like real leaves, simultaneously increasing in size.

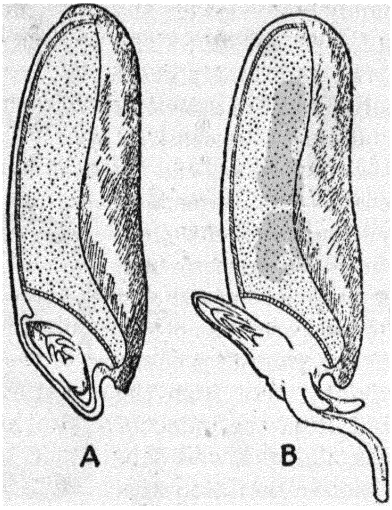


FIG. 22.—Grain of wheat in longitudinal section: A—previous to germination; B—in the beginning of germination.

The further growth of the embryo consists in an increase in size of all the initial organs of the embryo, the rootlet first, then the leaves and the stem. With the gradual enlargement of the seedling, it continually develops new organs that were absent in the seed, *viz.*, new leaves, roots, branches, stems, etc. Gradually the parts that have been formed earlier complete their growth. At least, they cease to increase in size, and in later stages of development in the plant, completely mature tissues and organs may be distinguished from the embryonic tissues that then appear at the ends of the roots and stems and that consist of continuously dividing and growing cells.

In the course of their growth, many plants, especially the perennials, differ essentially from animals. When the animal is

mature, its growth ceases. The annual plant may show a similar tendency. But in the perennial plant, growth continues throughout its life and consists in a continuous formation of new organs from special meristematic regions. Young tissue is always found even in trees several thousand years old; while, in an old animal, the entire organism in all of its parts is senescent. In the animal, only a few parts represent dead tissues; while in plants, old dead structures may still function for support, protection, and water conduction.

27. Growth of Separate Cells and the Processes Involved. Three Stages of Growth: Embryonic Development, Expansion, and Internal Differentiation.—Since the growth of separate organs as well as of entire plants is made up of the sum of the growth of the separate cells composing them, it is essential for understanding the laws of growth to study the alterations that occur during growth in the cells of the growing points and meristematic tissues. Meristematic cells are comparatively small, have very thin cell walls, and are filled with protoplasm with a large nucleus. Growth of meristematic tissues proceeds as follows. The amount of protoplasm in the cells gradually increases, resulting in an increase in size of the cells themselves; thereupon, division of the nucleus and of the cytoplasm takes place, and the newly formed cells are separated from each other by new walls. The accumulation of living substances begins anew, followed by a new division of cells. Consequently, in the meristematic regions, the fundamental process is an increase in the number of cells and in the general mass of living matter.

In organs with uninterrupted growth, such as roots or young stems, the total mass of meristematic tissues remains constant in spite of the continuous division of its cells. This is due to the fact that in the lower part of the growing region the meristematic cells pass into the second stage of growth, elongation. This stage is characterized by the appearance of vacuoles in the protoplasm, which become filled with the cell sap (Fig. 23, lower and central cell). The vacuoles enlarge rapidly, and finally the protoplasm is only a thin lining appressed to the walls. The vacuole of the cell has increased, the walls have grown considerably, but the amount of protoplasm apparently remains about the same as in meristematic cells (Fig. 23).

The enlargement of the cell during the stage of elongation is the result of causes different from those operating during meristematic growth. In meristematic cells, synthesis increases the amount of protoplasm; while, in elongating cells, there is an increase in the quantity of cell sap, consisting mainly of water. The question naturally arises: "What causes water to accumulate in the cell and to enlarge the cell volume?" The process

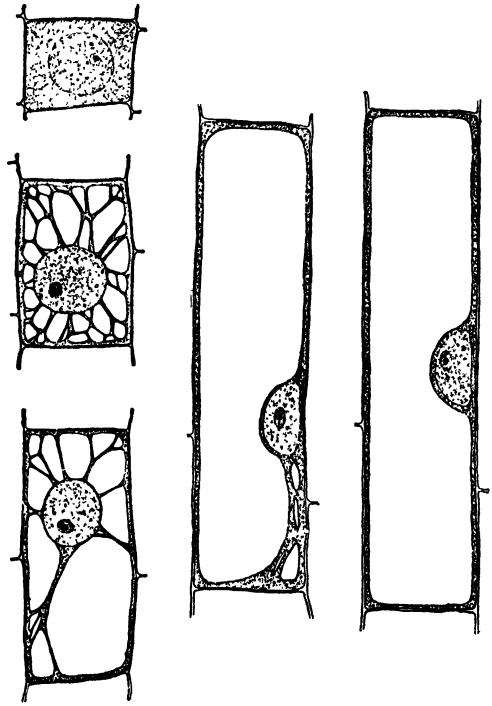


FIG. 23.—Successive stages in the growth of root cells (*after Brown*).

of cell elongation was considered for a long time to be as follows. It was supposed that there appear in the cell osmotically active substances like sugars or organic acids. These substances by their osmotic action cause water to move through the cell membranes, resulting in an increased turgor of the cell. The cell wall, still thin and elastic, will begin to stretch under the influence of turgor pressure, like a rubber balloon when air is pumped into it, and the volume of the cell increases. At the same time, there occurs a dilution of the osmotically active substances, resulting

in a decreased osmotic pressure within the cell and a gradual lowering of turgor pressure. The stage of elongation then terminates.

This idea, appealing in its simplicity and permitting one to compare the growth of living cells with artificial ones prepared from precipitation membranes, for a long time enjoyed great popularity. However, facts began to accumulate that contradicted it. It was found that osmotic pressure does not decrease in cells as they grow but remains fairly constant. Moreover, Ursprung's recent researches have shown that in the period of elongation, cells do not have a greater but, on the contrary, a lower turgor and at the same time a considerably increased suction tension, or tendency to absorb water. With the osmotic pressure unchanged, an increase in suction tension can take place only when there is a diminished pressure of the cell membrane, as has been explained in Art. 6. The diminished turgor pressure during the increase in volume of the cell may be the result either of a rapid growth of the cell wall or of an increase in its elasticity.

Ursprung's investigations enable us to construct the following mechanism of the growth of cells in the stage of elongation. The increase in amount of protoplasm, characteristic of the embryonic stage, is replaced by an augmented production of cellulose and other substances of the cell wall, thus causing the membrane to grow rapidly. The pressure of the wall on the cell contents now diminishes, and as a result there arises a considerable suction tension, causing water to enter the cell. This suction is so great that in case of water deficit, young growing parts may draw water from older or already mature cells. This explains the fact that young shoots of the potato and other tuber and root crop plants can absorb water from the storage tissues on which they develop, although the absolute degree of difference of their osmotic pressures is not great.

Elongation is followed by the third and last period of growth, the stage of internal differentiation. The thin, stretched wall now grows in thickness. The cell has ceased to increase in volume and assumes its final form and size. The homogeneous zone of meristematic cells gives rise to the various tissues of the mature organ, such as the conducting and mechanical elements. The internal factors that control differentiation, frequently

accompanied by the formation of special thickenings in the cell walls, pitting of the wall, the loss of the cell contents, etc., remain unknown at present; but the changes of cell structure in differentiation are associated with the assumption of a definite function by the cell. To perform its function, a cell takes on a special structure. Morphological changes always follow changes of physiological function.

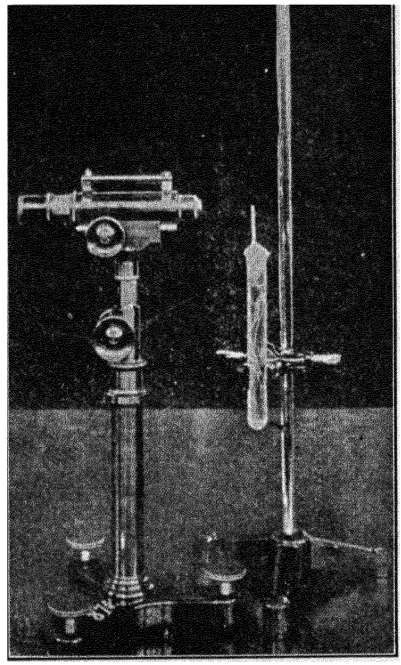


FIG. 24.—Measurement of growth by means of a horizontal microscope (*after Molisch*).

28. Methods of Measuring Growth. General Laws of Growth and Attempts at Their Mathematical Formulation.—An exact study of the phenomenon of growth requires, in the first place, a precise measurement of it. Several methods are available for this purpose.

The simplest of these consists in measuring the length of the growing organs or of whole plants at definite time intervals by means of a properly subdivided ruler. As the growth of plants on the whole is rather slow, this comparatively crude method

can be used only when measurements are taken not oftener than every 24 hrs.

When it is desired to follow the growth of a plant at shorter intervals of time, a horizontal microscope will be found convenient. It is focused upon the end of the growing organ, *e.g.*, upon the tip of a blade of grass or the end of a root; and then, by means of an ocular micrometer, the distance covered by the extremity of the plant is determined (Fig. 24). If the magnification of the microscope is known, it is easy to calculate the growth increase.

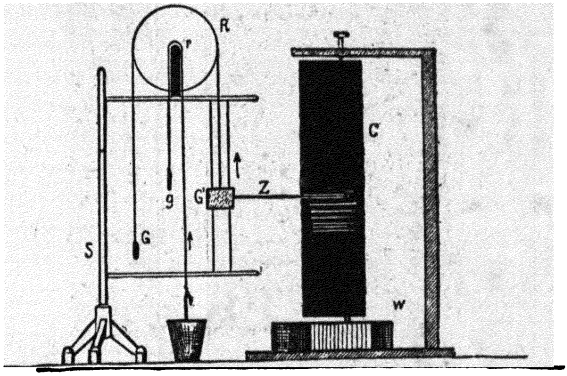


FIG. 25.—Wiesner's auxograph (*after Molisch*). Clockwork, *W*, rotates a cylinder *C*, covered with lampblack on which pointer, *Z*, marks the growth elongation shown by thread *g* passing over small pulley *r*. The sliding counterweight, *G*, attached to thread over the circumference of wheel, *R*, multiplies the movement.

It is very convenient to use for studies of the course of growth a self-recording apparatus, the so-called "auxograph," in which, by means of pulleys and wheels, changes in the position of the tip of a plant are recorded on an enlarged scale on the surface of a revolving smoked paper cylinder (Fig. 25). Auxographs usually give an enlargement of 20 to 40 times. From such records, one can read alterations in growth occurring in the course of ½ or 1 hr. Recently, however, the Indian scientist Bose has constructed an apparatus, the crescograph, which gives enlargements of 1,000 and even 10,000 times. This apparatus allows one to record the progress of growth not only by minutes but even by seconds.

Often it is important to follow the growth of certain organs instead of that of the whole plant. In such cases, Sachs's

methods of marking the growing organs by means of India ink applied with a fine brush is used. These marks are made at definite distances from one another, and the increase in the distance is measured at certain time intervals. This enables one to obtain a precise measurement of the changes in length of each portion during the experiment (Figs. 26 and 27) and to ascertain the dimensions of the entire growing zone, since only in this region will the distance between the marks increase.

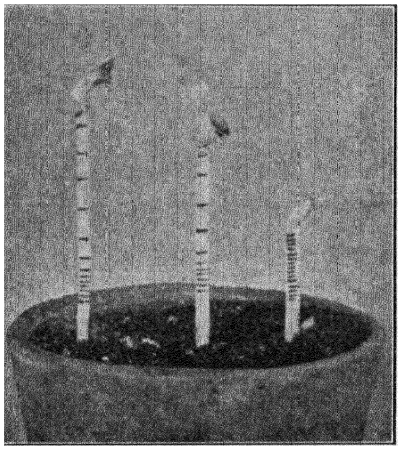


FIG. 26.—Two etiolated seedlings of the horse bean with markings made 24 hr. before photographing. On the right, a seedling with markings just made (*after Nathansohn*).

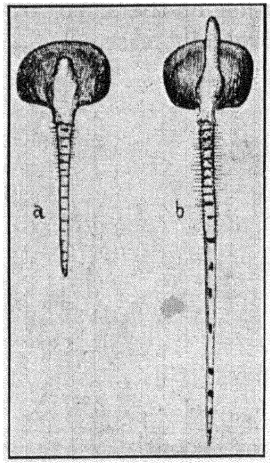


FIG. 27.—Maize sprouts with markings made on roots; *a*, before the experiment; *b*, 24 hr. later (*after Molisch*).

Investigations performed by means of this method have established differences in the character of growth of various organs of plants. Stems and roots grow at their ends while, in many leaves, growth is mostly at the base. Leaves are limited in their growth, very soon reaching their final dimensions; while, in stems and roots, growth continues throughout their life, in trees sometimes during hundreds and even thousands of years, thus permitting them to reach enormous sizes. Roots and shoots differ in that the growth of roots is limited to a very short section, not more than 1 cm. This allows the root to overcome the resistance of the soil it has to push through; while in the shoots, the length of the growing zone is up to 10 cm. long.

During recent years, wide use has been made of the photographic method for studying the course of growth. At definite

time intervals, the growing parts are photographed, and all the measurements are carried out on these photographs. They may be used also for motion-picture projection of the growth phenomena. The photographic method possesses the advantage of permitting measurements of any degree of precision without the growing organ being touched, all disturbances of its normal development thus being eliminated. The drawback of this method is the necessity of applying light. Light, as will be seen later, greatly affects growth. Therefore, in cases when the effect of light must be eliminated, the photographs must be made in red light, which affects growth very little.

On the basis of growth increments of separate regions as well as whole organs, Sachs established a definite law of the "grand period of growth." According to this law, every organ, and each definite region of a developing organ, at first grows slowly, then accelerates, reaches its maximum speed, and finally slows down. The following figures show daily growth increments of a small section in the growing roots of the horse bean, which at the beginning of the experiment was 1 mm. in length.

Days	0	1	2	3	4	5	6	7	8
Growth increase, millimeters		1.8	3.7	17.5	16.5	17.0	14.5	7.0	0.0
Total length, millimeters	1	2.8	6.5	24.0	40.5	57.5	72.0	80.0	80.0

In the bottom row are given the figures of the whole length of the region at the time of its measurement. If the results of these measurements are represented graphically, two distinct curves are obtained (Fig. 28). The curve of the rate of growth has an almost symmetrical aspect with a rather prolonged maximum (dotted lines), while the curve of changes in the total length of the section is of the letter S type. It rises, at first slowly, then more rapidly, passes through a point of inflection at the time of most rapid growth, and finally approaches the horizontal direction.

In this growth curve, one can distinguish two sections: from the beginning to the maximum, or the most rapid rise; and from the maximum to the cessation of growth. The first part may be termed the "initial phase," since here is the manifestation of growth in its simplest aspect. The second part may be con-

sidered as the development of retardation, which shows itself at first weakly but later with increasing importance, until growth ceases. For the initial phase, Blackman applies the mathematical formula for calculating compound interest, since, in growth we are dealing with an increase in the number of cells following a geometrical progression, in which one cell forms 2, 2 producing 4, then 8, 16, 32, 64, etc. Designating by the letter A the final size of the section, and by a, the initial one; by t, the time interval; and by r, the rate of percentage increase: the formula $A = ae^{rt}$ is obtained, where e is the base of natural logarithms. Changing this formula to a logarithmic form and converting the natural into decimal logarithms, we obtain

$$2.3026 \log \frac{A}{a} = rt,$$

whence it is easy to compute the percentage of growth per day, the initial and final size and the number of days being known.

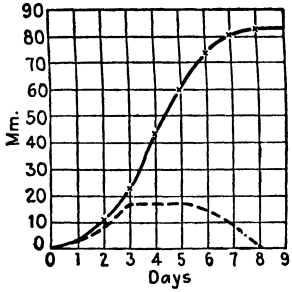


FIG. 28.—Growth curves of roots of *Vicia faba* (*after Rippel*).

Blackman's formula is applicable only in a limited way to the initial phase of the curve, for the exact moment of retardation is very difficult to determine. Growth is based on the chemical transformation of assimilated or reserve substances into living protoplasm, and the latter begins to participate in the synthetic reactions as soon as it is formed. Robertson has made an attempt to apply to growth the formula of monomolecular autocatalytic reactions, which should embrace the entire process from beginning to end. If one considers the gradual retardation of the reaction as being due to the accumulation of its end products, which does not always take place because of their rapid removal, then the formula may be represented by

$$\frac{dx}{dt} = K(A - x),$$

where K is an empirical constant; x, the size reached in t days, or other time units, from the beginning of growth; and A the final size of the growing organ. By integrating and other trans-

formations of the equation, Robertson deduces from this formula another, which is more convenient for calculation:

$$\log \frac{A - x}{x} = k(t_1 - t_0),$$

in which $(t_1 - t_0)$ represents the time necessary for the growing organ to reach one-half its final size. For many calculations, it is convenient to take relative values instead of absolute ones, and to assume the final value of A to be 100.

Besides Blackman's and Robertson's formulas, others have been proposed. But all of them fail to express adequately the progress of growth, since, in addition to internal conditions, the increments per unit time depend also on environmental factors, such as temperature, light, and supply of nutrient substances. Consequently, these formulas represent the process of growth only approximately, Robertson's formula having the advantage that it attempts to cover the whole period of growth.

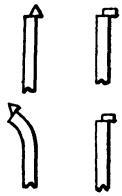
The causes that retard and check growth are not fully understood at present. Here one must consider the problem of the effect of certain environmental factors, specific internal catalysts, and the general senescence of an organism, which is still unsolved.

29. Growth Hormones, Auxin, Its Action and Chemical Composition.—The growth of cells in the zone of elongation depends most intimately upon the activity of the zone situated above it. If the top of the growing stem is cut, the elongation of the zone situated below it will be retarded and accelerates again only in case processes of regeneration occur on the cut end. Such an inhibition in growth can be clearly observed on peduncles after removal of the flower bud, and on the coleoptile of cereals protruding from the soil a few centimeters.

The coleoptile represents the first leaf of grass seedlings. It is colorless and possesses the shape of a hollow cylinder passing at the tip end into a complete cone. It is similar to a finger of a glove and serves for boring through the soil that covers the seed and protecting the tip of the first green leaf that grows up through its cavity. The growth of the coleoptile is mostly at its base. It is very rapid at the beginning, being greater than the growth of the first leaf. Later on, its growth slows down; and then the first leaf overtakes it, ruptures the tip, and appears outside. After this, the growth of the coleoptile ceases completely.

Boysen-Jensen (1910) observed a fact of great importance, that growth of a decapitated coleoptile may be appreciably hastened if the cut tip is stuck back on the cut end by means of a drop of water or gelatin.

However, if a thin piece of tin foil or mica is placed between the cut end and the tip, acceleration will not be observed. Hence Boysen-Jensen drew the conclusion that the tip of the coleoptile excretes a special substance that accelerates growth and is capable of diffusing through a layer of water or gelatine.



1 2

FIG. 29.—Bending of the oat coleoptile under the influence of auxin, 1, contained in an agar block, compared with agar alone 2.

This stimulating effect may be easily demonstrated if the cut tip is placed on one side of the cut end. This side will obtain more of the growth-promoting substance than the opposite one and will grow more rapidly. In consequence, the seedling will curve toward the opposite side (Fig. 29). Such a curvature is certainly much more easy to perceive than the general steady increase in length.

The development of the ideas of internal secretion in animal physiology and the isolation of a series of animal hormones contributed greatly to further advancement in the concept of growth-promoting substances. At present, the term "hormone," introduced to science in 1905 by the animal-physiologist Starling, designates a fairly large group of substances elaborated by the organism and possessing the capacity of directing and regulating the course of different vital processes. In the animal organism, the hormones are produced by special glands of internal secretion, endocrine glands, and are introduced into the lymphatic and blood system, which carries them throughout the body. The most widely known of the hormones are *adrenalin*, excreted by the suprarenal gland; *insulin*, secreted by the pancreatic gland; *folliculin*, elaborated by the ovaries; and many others. In their chemical structure, the hormones do not show much chemical relationship, except that a number are related in structure to phenanthrene. In common with enzymes, they possess a very high activity at exceedingly low concentrations, but they are distinguished by a greater stability in respect to heat and other destructive agents.

The explanation of growth-promoting substance, or growth hormone, has advanced especially rapidly during recent years, chiefly in connection with the work of the Dutch physiologist Went and his coworkers, and of Cholodny in Russia. To obtain the growth hormone in quantities sufficient for obvious display of its activity, Went applied the following procedure. The cut tips of oat and corn coleoptiles (the tips of these two plants are the most active) about 1 to 2 mm. in length were placed on a thin lamina of agar gel and remained thus for about 1 hr. After this, the lamina was cut into separate small cubes, each of which contained a certain amount of the hormone (Fig. 30). Such blocks, when placed eccentrically on decapitated seedlings,

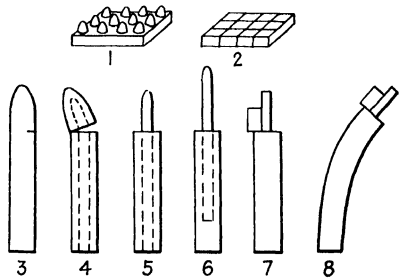


FIG. 30.— Method of extracting and measuring auxin. 1, plate of agar with 12 coleoptile tips; 2, tips removed, agar divided into 12 cubes; 3, coleoptile with incision; 4, 5, decapitated coleoptiles; 6, first leaf partially pulled out; 7, one of the agar cubes placed on one side of the coleoptile; and 8, result, bending of the coleoptile.

provoke a growth curvature. The amount of hormone sufficient to produce a curvature of 10 deg. in an oat coleoptile was defined as 1 "oat unit." Such blocks impregnated with the growth hormone were used for studying some of its properties. It proved to be a fairly stable substance, not destroyed by boiling. The rate of its diffusion through agar-gel was used to determine its molecular weight, which proved to be about 350.

Growth hormones may be formed not only in tops but also in several other parts of the plant organs. Thus according to the experiments of Cholodny, the substance is formed in definite elements of the phloem in hypocotyls of lupines and sunflowers. If by means of a special borer the central cylinder with all its elements is removed from a cut seedling, the growth rate is appreciably inhibited. Now if the cut tip of a corn coleoptile is

placed in the center of such a hollow stem, growth of the stem will be considerably accelerated under the influence of the growth hormone excreted by this tip.

These experiments show that the growth hormone is not specific; the excretion of the tip of the corn coleoptile increases the growth rate of stems of the lupine and sunflower, and the head of the dandelion can accelerate the growth of the peduncle of the poppy or the coleoptile of oats. The growth-promoting substance is capable of moving only in the direction from the morphological top to the base, independent of the orientation of the organ in space. According to Went, the mechanism of its activity consists in the softening of the cell walls, which facilitates their expansion. This hormone is utilized in the process of growth, and the lower-lying zones are provided with smaller quantities than those situated higher. This explains the distribution of growth in the elongating organ, the grand period of growth that has been described in the foregoing article.

It is interesting to note that the root tip excretes a substance that does not accelerate but inhibits growth of the root zone. This is dependent not upon the properties of the substance but on the peculiarities of the cells of the zone of elongation. Experiments of Cholodny (1927) have shown that cut tips of roots accelerate the growth of decapitated stem bases, and, conversely, that tips of stems inhibit the growth of decapitated roots. Thus the growth hormone proves to be identical, but various organs react differently to the influence of this substance.

The presence of the growth hormone may be revealed not only in the tops of plant organs but likewise in other parts of the plant and in secretions of the animal organism, *e.g.*, in the saliva. But the discovery of this hormone in the urine of man and animals was of special significance, for it permitted the Dutch chemist Kögl to obtain it in quantities sufficient for detailed chemical study. In its preparation, he made use of its solubility in ether and insolubility in benzene, its capacity of giving insoluble salts with lead and soluble salts with calcium, etc. After a series of complicated manipulations, Kögl finally obtained 250 mg. of a completely pure crystalline hormone, which he designated "auxin," and established its chemical nature. It proved to be a monobasic acid of the empirical formula, $C_{18}H_{32}O_5$, easily transforming, when stored, into an isomeric completely inactive

form. Urine contains on the average about 2 mg. of auxin per liter. Subsequently, several auxin-like substances have been identified, of which indole-3-acetic acid is the one most widely used in experimental and practical work. It stimulates roots and causes callus and gall formation on stems of plants.

The physiological action of pure crystalline auxin is very intense. According to Kögl, 1 mg. of auxin contains about 50 million oat units, thus corresponding to an amount of hormone contained in 7 million coleoptiles of corn, for each such tip contains about 7 oat units.

Auxin gets into urine probably from the plants used for food. It is especially abundantly excreted when large amounts of vegetable oils are consumed. But it is probable that bacteria inhabiting the digestive tract also participate in its formation; for substances similar to auxin were found among the products of the vital activity of different microorganisms, especially of bacteria and molds.

The growth hormone is capable of moving through the plant only in one direction, from the proximal end toward the base, and never moves in the reverse direction from the base to the top. According to Cholodny, this is connected with the distribution of the electric potentials in the stem, the top being charged negatively in relation to the base. That is why the anions of auxin, representing a weak acid, must move cataphoretically within the stem in the direction of the positive pole, *i.e.*, to the base. This opinion is confirmed by the fact that a vertically standing coleoptile of oats, placed between electrodes with a high potential difference between them, curve in the direction of the positive pole, this being easily explained in the following manner: The side of the coleoptile turned to this pole charges negatively through an induced charge; and the flow of the hormone is directed to the opposite side, promoting an acceleration of its growth.

Auxin produces an accelerating effect only upon the stage of elongation and does not influence the division of cells, which determines the stage of meristematic growth. According to some authors (Haberlandt, Cholodny), plants possess specific hormones that excite cell division. These hormones are elaborated in the phloem cells (leptome) of the conductive tissue and have therefore been designated "leptohormones." Their activ-

from excessive heat, may allow the development of disease and may even cause the death of an organism.

Within the inhibitory ranges, there is no growth; but the other vital processes, such as respiration, assimilation, and metabolism, may still continue. The cause of this check in growth must be looked for in the disturbed harmony between the separate functions of the plant, since growth is a very complex process requiring a precise coordination of all functions. The rate of the various metabolic processes, however, changes in different degrees with variations of temperature. For instance, respiration increases until a lethal temperature is reached, its maximum and optimum coinciding; while assimilation decreases considerably earlier. Therefore, at temperatures exceeding the required optimum for assimilation, consumption will overbalance assimilation, and the plant will soon be exhausted.

The optimum temperature for growth is by no means the most favorable one for the general development of a plant. According to the usual definition, the optimal point is one at which growth proceeds most rapidly. But the highest rate of growth is not always the most desirable for the development of a strong and healthy plant. Because of the rapid consumption of food materials, plants that show fast development or, as gardeners would say, have been forced at a high temperature are usually weaker than those that have developed at a lower temperature. A purely physiological optimum, therefore, indicating the greatest rapidity of development, must be distinguished from the harmonious optimum, producing the most vigorous plants.

It is evident that the exact determination of such an optimum presents greater difficulties than the estimation of the physiological optimum. It requires, in the first place, much technical equipment, such as several greenhouses where temperatures can be regulated with precision. This, however, is available only in a very few scientific institutions. Observation of plants in their natural state usually shows that the temperature optimum for growth is not constant for the whole period of development of a plant. It varies continually from germination to flowering and to maturity. For most annual plants, at least, a general rule can be established that, in the earlier stages of development, this optimum lies at a lower point than during the later ones. This coincides with the natural march of temperature from spring to

early fall. A temperature too high at the beginning of growth is one of the chief causes of the development of weak plants yielding a poor crop. This commonly results from late sowing. This rule, however, cannot be applied to thermophilous plants of tropical regions.

Certain plants may show a pronounced need for low temperature. Such are the winter cereals, which are usually sown in autumn and, after a prolonged winter period, produce seed during the following summer. If sown in the spring, they will tiller throughout the summer without reaching the fruiting stage. Formerly it was thought that winter plants require a rest period. Later, however, it was found that if winter cereals were planted after having germinated at a temperature about 0°C., heading could be obtained the same year without any inhibition in development. Winter cereals, therefore, may be said to require cold in the early stages of their development (Gassner, Lysenko, Maximov). Instead of allowing them to germinate in the cold, it is better to keep seeds of winter cereals that have just started germination for a prolonged period at a low temperature; this method makes it possible to obtain a yield the first year.

This method has been given the name of "jarovisation," or "vernalization," of winter crops. Further investigations of Lysenko have shown that the requirement of a low temperature in the beginning of development applies not only to winter cereals but also to many summer crops. Such crops, in passing the stage preceding reproduction, designated by Lysenko the "stage of jarovisation," require during a definite interval of time a certain temperature. The method of jarovisation elaborated by Lysenko has been given wide application during recent years in agricultural practice in Russia, especially in case it is important to accelerate the development of plants, *e.g.*, in arid and in polar regions. As this method is most intimately connected with ideas of the stages of development of plants, this question will be considered further in Chap. XII, which is devoted to problems of development and reproduction.

31. Influence of Light on Growth. Daily Periodicity of Growth. Formative Influence of Light.—In contradistinction to temperature, light is not a factor necessary for mere growth. Practically all plants can grow in darkness, while some plants, such as the bacteria and many fungi, can complete their whole

development in absolute darkness without essential alteration to the character of their growth. Higher plants, at least their green organs, usually show in the absence of light a rather marked divergence from their normal form and are lacking in green color. Such plants are said to be etiolated.

Etiolated plants differ from normal ones mainly in having excessively elongated stems and underdeveloped leaf blades (Fig. 32). In case, however, the stem does not grow during the first stages of development (*e.g.*, in the case of grasses), a stretching of leaves takes place. This faculty of elongating its organs in darkness is of great importance in the life of a plant. Finding itself deep under the ground, it stretches its shoots till they have reached light and then begins to unfold its leaves. Anatomically, etiolated plants are distinguished by tissues that are very little differentiated and by the prevalence of primary parenchyma. Besides, they are devoid of chlorophyll and are usually of a pale yellow color.

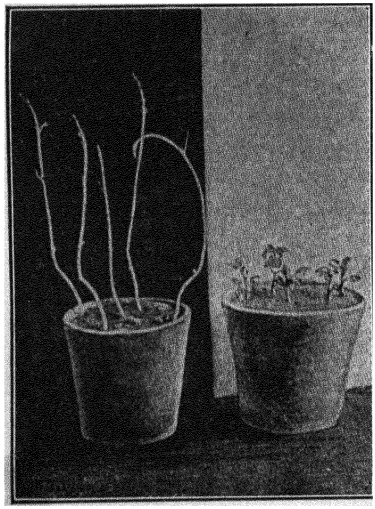


FIG. 32.—Etiolated (*l*) and normal (*r*) seedlings of the broad bean.

There is no direct connection between etiolation and the absence of chlorophyll or the shortage of nutrients. Stretching in darkness is observable in many fungi, *e.g.*, *Mucor*, *Pilobolus*, *Coprinus*, etc., while very often no sporangium (*Mucoraceae*) or pileus (*Basidiomycetes*) is formed, or else these are underdeveloped. Elongation in darkness is likewise pronounced in sprouts of the potato, which are abundantly supplied with nutrient substances from the tuber. In spite of numerous attempts to explain the causes of the peculiar alterations in form of etiolated plants, knowledge of the subject remains very scanty. Light evidently is necessary to produce a normal cell structure and tissue differentiation. Violet and ultraviolet wave lengths retard cell elongation. Growth in an atmosphere saturated with water vapor also leads to poor development of leaves and elongation of

stems as well as retarded differentiations of tissues. Hence, many authors, Palladin, for instance, are inclined to explain, in part at least, changes in the form of etiolated plants by curtailed transpiration in the absence of light (Art. 69). Recent observations, however, have shown that even short daily exposure to light, 5 to 10 min., from a rather weak source, is sufficient to induce etiolated plants to develop a normal form. This occurs in spite of the fact that in so short a time transpiration is not able to change to any extent, nor assimilation to begin, since little

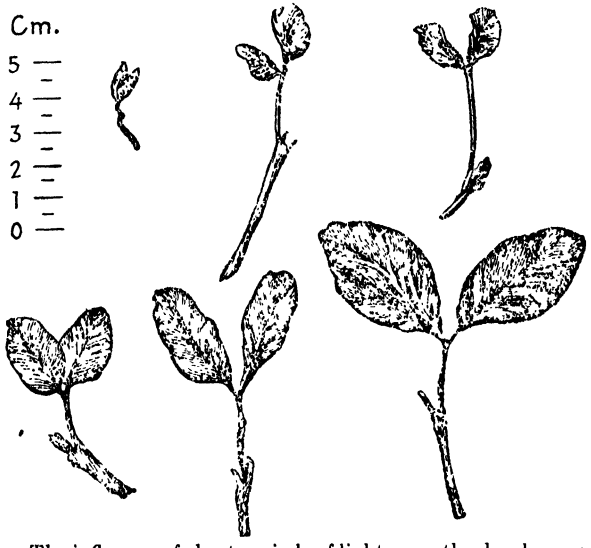


FIG. 33.—The influence of short periods of light upon the development of bean leaves. Upper row: left, from plants kept in absolute darkness; center, under 1 min., right, 5 min. of light per day. Lower row: left, under 10 min.; center, 60 min.; right, under full daylight.

chlorophyll is formed (Fig. 33). It is probable that under the influence of light there arise in the plant some substances of the nature of hormones, which lead to changes in the character of growth of leaves and stems. This assumption is confirmed by the fact that violet and ultraviolet rays are of the greatest effect in altering form; while the red part of the spectrum, which increases assimilation and transpiration, in this case produces the least effect (Fig. 34.).

Light has also a considerable influence on the rate of growth of plants. It retards growth in a definite way; and the higher its intensity, the greater the retardation. Moreover, under the

influence of light, plants pass more rapidly through the grand period of growth, the period of elongation terminating very early, even before the growing organs have had time to elongate. Consequently, shoots that have developed in strong light are always shorter than those grown in shade.

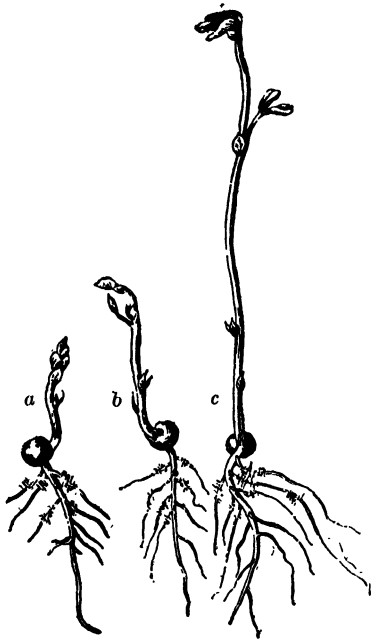


FIG. 34.—Seedlings of peas grown for an equal period (*a*) in white, (*b*) blue, and (*c*) orange light (*after Duggar*).



FIG. 35.—*Betonica* plants, *P*, grown in the valley, and *M*, upon a mountain (*after Bonnier*).

The behavior of the seedlings at the moment they have pushed their way through the earth and emerge into light is very interesting: their growth is instantly checked. This affects equally all parts remaining in the soil. Under the influence of the light, there evidently arises in the tip some growth-inhibiting stimulus that is transmitted to the underground parts. Some authors hold that this stimulus is a special growth-inhibiting hormone

that diffuses from the top downward. Others think that light retards the synthesis of the growth-promoting substance necessary for the elongation of cells.

The dwarfing effect of light on the aerial organs is particularly marked in high mountains, where, on account of greater transparency of the atmosphere, solar light is considerably richer in ultraviolet rays than in valleys. Alpine plants, as the investigations of Bonnier have shown, are always of short stature. And when common plants of the valley, *e.g.*, the dandelion or *Betonica*, are grown in high mountains, they assume the characteristics of alpine plants (Fig. 35). The retarding influence of light on growth is so great that it creates a definite daily periodicity. During the night, plants grow more rapidly than during the day, in spite of the lower nocturnal temperature. A very sharp drop of temperature may overbalance the accelerating influence of darkness, a condition leading to stunted plants, as exemplified by the vegetation of the tundra. Low temperatures at night have an influence also on the growth of alpine plants, adding their effect to the influence of bright light in producing increased dwarfing.

In prolonged experiments with green plants, the growth-inhibiting influence of light may be masked by its indirect effect. Being a source of the necessary energy for the production of organic matter, light promotes nutrition and consequently better growth of plants. Therefore, in well-lighted places, plants generally develop better than in shaded ones. Plants will grow better in a room when kept near a window than when farther back from it, where they may become exhausted. The light requirements of plants vary greatly, and a light intensity that is insufficient for some plants may be excessive for others.

32. Influence on Growth of the Degree of Saturation of the Plant with Water.—Normal growth processes require saturation of protoplasm with water; for only in a sufficiently mobile protoplasm can there be the free movement of the chromosomes necessary for the process of mitosis and other functions accompanying the division of cells. The stage of elongation is still more dependent upon the conditions of water supply, for the increase in volume depends upon the absorption of water by the cell. An excellent example of the significance of the degree of saturation with water for growth is presented by seeds. Air-

dry seeds contain only hygroscopic water in equilibrium with the ordinary atmosphere, when the moisture content is usually about 10 to 12 per cent of the dry weight. Such seeds may remain during many years without any signs of germination and produce carbon dioxide in very low amounts. But as soon as they are soaked in water, they begin to germinate, provided that other necessary conditions are adequate, such as the temperature and the aeration. If the seeds are given not the 50 to 60 per cent of water necessary for their complete saturation but only about half of this quantity, the process of respiration, as well as the beginning of hydrolytic decomposition of the reserve substances, will be perceptible, but growth will not take place. Such an incomplete excitation of the vital activity from partial moistening of the seeds is of great importance theoretically. It proves that various vital processes require a different degree of saturation with water, the greatest amount being necessary for growth. In general, much water is required for all functions connected with the formation of new masses of living substances, and considerably less for respiration and for digestion of the food reserves of the seed.

Walter (1931) designated the degree of saturation of the protoplasm with water by the term "hydrature." The amount of water is determined not so much by the total water content of the protoplasm or of the cell, expressed in percentage of the dry weight, as by the force with which the cell absorbs water from the surrounding medium. Walter measured the degree of saturation by the vapor pressure at the surface of the cell or other bodies impregnated with water. When completely saturated with water, the cell will be in equilibrium with an atmosphere saturated with vapor, and its hydrature may be expressed as 100 per cent. When placed in an unsaturated atmosphere, a moist body will lose water, but simultaneously its water-holding capacity will begin to increase. If the vapor pressure of the surrounding atmosphere is 95 per cent, the moist object may cease to lose water only after an increase of its water-retaining capacity up to a magnitude that corresponds to the vapor pressure of a solution with an osmotic pressure of 55.8 atmospheres, at a temperature of 20°C. It is well known that an increase in the concentration of a solution is accompanied by a corresponding increase in its osmotic pressure and a decrease in the vapor pressure at

its surface. These two magnitudes are connected in a definite relationship; *viz.*, 1 per cent decrease in the vapor pressure corresponds to an increase of approximately 13.5 atmospheres in osmotic pressure.

The connection between growth capacity and the degree of saturation of the surrounding atmosphere is most obviously manifested by lower organisms, the cells of which when active are not protected against water loss into the surrounding medium and consequently must be in hydrostatic equilibrium with the latter. According to the observations of Walter, many molds begin to retard their growth at 98 per cent relative humidity of the atmosphere, and at 95 per cent, growth is completely stopped. Only the most resistant species can continue growth in a medium where the humidity falls to 90 or 85 per cent relative humidity; this corresponding to an osmotic pressure of about 127 to 217 atmospheres.

In higher plants, the cells of the growing point of the roots do not have protective coverings and are entirely dependent upon the hydrature of the surrounding medium. For this reason, roots are capable of growing only in a sufficiently moist soil, whose interstices contain air almost completely saturated with water vapor and where the osmotic pressure of the soil solution does not exceed 10 to 15 atmospheres. In a dryer soil, root growth of most plants is not possible, and only roots of some of the desert plants that are provided with protective tissues are capable of pushing through dry soil.

Aerial parts of the plant almost always remain in a comparatively dry atmosphere, the relative humidity of which falls at midday to 40 to 50 per cent and frequently considerably lower. The growth of these parts is possible only for the reason that the meristematic tissue of the growing points is protected from direct contact with the dry atmosphere by the closely packed leaves of the bud, while the lower zone of elongation is covered by an epidermis with a cuticle impermeable to water. A similar epidermis covers the leaves, and owing to its presence the intercellular spaces adjacent to the living cells of the growing leaf tissue possess an atmosphere of not less than 99 to 98 per cent relative humidity.

As every increase of the cell-sap concentration involves a corresponding withdrawal of water from the protoplasm, a close

correlation may be observed between the concentration of the cell sap and growth. This is especially obvious in the growth of tree shoots.

In spring, the root system delivers abundant water, and, evaporation being insignificant, there results a vigorous growth of the shoots. With the development of the leaf surface, evaporation begins to overtake the delivery of water, the concentration of the cell sap increases, and growth first decreases and then stops. In general, with a deficiency of water or with accumulation in the soil of substances that inhibit the water supply, the stage of elongation terminates too early, and differentiation begins before the cells have reached their full size. This leads to the production of small cells and stunted plants. The same result is obtained when the water loss is too rapid. Strongly evaporating leaves may lose water faster than it is delivered from the soil; consequently, they withdraw water from the growing organs and disturb most of the tissue in the zone of elongation, and this leads to the formation of small cells and to a diminution in the size of the entire plant.

In annual cereals, to which belong our most important crops, such as wheat, rye, barley, and oats, the developing stem shows a peculiar growth distribution. First, only meristematic growth takes place in the stem, and only after all its internodes, as well as inflorescences, have been laid down, does elongation or the so-called "shooting" begin. During this period, the plant is especially in need of an ample water supply for the growing parts, and, hence it is sensitive to any deficiency. Field observations have shown that an insufficient rainfall during the period immediately preceding shooting greatly reduces the yield of cereals. That is why this growth phase has been designated a critical period in relation to moisture (Brounov).

The most favorable conditions for growth are an ample moisture supply both as soil water and atmospheric humidity during all periods of development of a plant. Consequently, the greatest vigor and the highest rate of growth are attained by vegetation in warm, moist, tropical countries.

Artificial irrigation applied in many arid regions, *e.g.*, in the western United States and in Middle Asia, exerts a great influence upon growth. Irrigated plants are always considerably higher than nonirrigated ones, develop a larger leaf surface, and corre-

spondingly increase the yield. The osmotic pressure of the cell sap of plants after irrigation diminishes appreciably.

33. Influence of Chemical Stimulants on Growth. Toxic Action of Pure Neutral Salts. Balanced Solutions. Chemical Stimulation of Growth.—In considering the influence of various external factors on growth, it is necessary to keep in mind the following points. Growth is the result of complex chemical and physical processes, proceeding in the cell under continuous expenditure of energy, which is liberated through respiration. Growth is closely connected with respiration and ceases as soon as a plant is transferred to an oxygen-free medium. Other external influences, with the exception of temperature, which regulates the rate of all vital processes, and water, by which the material for enlargement of cells is supplied, act upon growth not directly but only indirectly, by producing certain deviations of the processes on which growth depends. External factors in nature, both chemical and physical, may act as growth stimuli. Their characteristic peculiarity is the lack of relationship between the quantity of the stimulant and the effect produced. Sometimes a feeble stimulus may produce a strong effect, and conversely, sometimes a strong stimulus is almost without influence on growth. The effect depends largely on the character of the stimulating agent and on the nature of its action on the normal course of growth. The phenomena of irritability are often compared to disturbances produced on a machine. Sometimes a touch, which may control some important lever, can completely change the movements of the machine, while a heavy shock applied to other parts may produce no effect. Since it is not known as yet what levers in the living machine, the protoplasm, respond to the action of certain stimuli, it is not surprising that these phenomena appear to be very complex and that in studying them very contradictory experimental results have been secured.

The effect of one of the physical stimulants, light, has been discussed already (Art. 31). The influence of other stimuli, chiefly of a chemical character, will now be considered. The foremost place must be assigned to the action of cations and anions.

In discussing the colloidal state, it has already been noted that the presence of electrolytes is absolutely necessary for the changes in the state of colloids that continuously occur in the cell. Thus

cells require for their growth the influence upon their growing organs of various electrolytes, in the first place, of cations such as potassium, magnesium, calcium, and of several others. The electrolytes necessary for plant growth will be discussed in greater detail in the chapter devoted to the mineral nutrition of plants. At present, there will be mentioned only the fact, of great importance, that each of the salts that in combination with other salts is indispensable for plant growth becomes toxic to plant cells when it is supplied alone in a pure solution containing no other admixtures.

This poisonous effect of single salts was first observed in experiments on mice (Ringer, 1883) and on sea animals (Loeb, 1898). Solutions of sodium chloride, corresponding in concentration to sea water, were found to have a pronounced toxicity if very pure salt was used. But small quantities of calcium and magnesium prevented this toxic effect. In a pure solution of sodium chloride, for instance, not a single embryo developed from the fertilized eggs of the sea urchin. But the addition of 1 cc. of a very dilute solution of $CaSO_4$ caused the development of 3 per cent of the embryos; 2 cc., 20 per cent; and 4 cc., 75 per cent. Pure solutions of calcium salts also are toxic, but this can be counteracted by the addition of sodium salts. Thus, different salts destroy the toxic effect of others. This phenomenon has been termed the "antagonism of ions."

Ion antagonism can be easily observed in the development of roots of plants in water cultures. It has been studied in detail by Osterhout. Figure 36 shows the results of one of his experiments. In pure solutions of $CaCl_2$ and of NaCl, the roots have developed very poorly. Much better growth was secured in solutions where Na^+ was balanced by Ca^{++}, but the best development was obtained when a third cation was added, *viz.*, K^+. The greatest antagonism is usually observed between univalent and bivalent cations, but ions of the same valence may be antagonistic. In general, the higher the valence of the ion, the smaller the concentration required for antagonistic effect.

By choosing different concentrations of the various ions, a combination may be obtained that will suit best the development of an organism. Such an optimal combination has been termed a physiologically balanced solution. For animals, as well as for marine algae, a balanced salt solution corresponds closely to sea

water, which approaches the proportional composition of salts in the blood and lymph. This caused Loeb to say that we all carry in our bodies a part of the ocean, where once animal life originated. A balanced medium for plants is represented by the solutions that are used for water cultures; a number of optimal mixtures have been described by Shive, Tottingham, and others.

In studying the effect of different neutral salts upon growth, it is very difficult to separate their participation in the structure of protoplasm from their influence as specific stimulants. The phenomenon of stimulation may be observed best in its pure effect by applying poisonous substances.

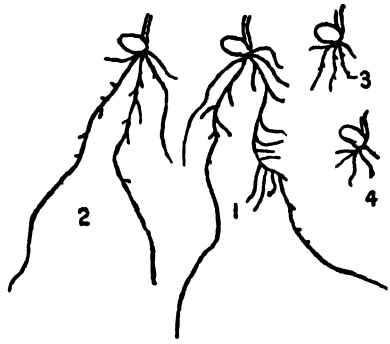


FIG. 36.—The comparative growth of roots of wheat: (1) in a balanced solution of NaCl + KCl + $CaCl_2$; (2) in a partly balanced solution of NaCl + $CaCl_2$; (3) in a pure solution of $CaCl_2$; (4) in a pure solution of NaCl (*redrawn after Osterhout*).

By poisonous substances are understood chemicals that even in small doses inhibit growth and in considerable quantities will kill a plant. Such, for instance, are the salts of heavy metals, copper, lead, silver, and mercury, as well as many organic substances like ether, chloroform, toluol. Certain metabolic products of an organism also may be poisonous, such as the organic acids, notably oxalic and carbonic acid.

A detailed study of the influence of poisonous substances on growth usually shows that not only the severity but the character of their action depend on their concentration. When applied in very weak doses, the majority of even the most poisonous substances will not depress, but stimulate, growth. If for purposes of calculation, a solution of 1 gram molecule per 100,000 l. of water is assumed as unity, then phenol, for example, in a concentration of 100 to 200 units will stop the growth of plants in water cultures, but it will have a stimulating effect in concentrations of 4 to 8 units. Ethyl alcohol, a weaker poison, stimulates in a concentration of 25 to 75 units and checks growth in concentrations of 2,700 to 7,500 units. A similar stimulating effect is produced by mercury preparations, which are widely

applied in the practice of treating seeds to protect them against fungi.

It is interesting to note that the effect of a poison depends also on the properties of the medium in which a plant is growing. The poisonous quality of many substances markedly decreases in sand cultures in comparison to water cultures, while in soil the plant is able to endure several hundred times as large a dose as in water. This is due to the adsorptive capacity of the soil, primarily the clay portion, which binds the poisonous substances on the surface of its particles.

The action of poisons does not differ essentially from that of the mineral salts, since in strong concentrations these too have an inhibiting effect on growth. But they can also stimulate it, if applied in weaker dilutions. The difference must therefore be considered to be quantitative rather than qualitative, and only substances that inhibit growth in very weak concentrations should be regarded as poisonous. It has been seen already that all cations show a toxic effect in pure solution and that only solutions that are physiologically balanced are suitable for the nutrition of plants.

Recently, the question of stimulants for growth has been given a new interpretation in the works of Popoff, who is of the opinion that it is not absolutely necessary that the stimulant should act continuously upon the plant during its development. In order to increase growth, it is sufficient to stimulate the germinating seeds. For this purpose, Popoff uses 3 per cent solutions of magnesium chloride and sulphate and of manganese sulphate and nitrate, as well as mixtures of these salts, and a number of organic compounds, such as tannin and various narcotic and antiseptic substances. The seeds are soaked in these solutions for several hours and then sown in the usual way. According to Popoff, as a result of such a treatment, growth has been accelerated, the size of the plants has increased, and considerably larger crops have been obtained. In some of his experiments, this increase has amounted to as much as 50 to 100 per cent.

Popoff's experiments have attracted a great deal of interest, since by the stimulation of seeds a considerable increase in yield may be obtained at little expense. But most investigators have not been able to confirm his results, especially under field conditions. Stimulation of seeds is a considerably more complicated

affair than it appeared at the outset. The conditions under which the experiments succeed have not as yet been determined.

Among the chemical stimulants, carbon dioxide, the normal product of respiration, occupies a singular position. Its accumulation in the atmosphere surrounding the growing organs causes a retardation of their growth and later checks various other vital processes, which after some time may be renewed. Thus, under the influence of carbon dioxide, the plant passes into a dormant state. To increase their storage life, fruits and vegetables are placed in an atmosphere of carbon dioxide in closed containers. This checks the development of molds, the chief enemies of fruit during storage, and retards respiration. These storage methods are applicable however only to such organs of plants as possess a low vital activity, such as fruits and vegetables. This method is of commercial value in the storage of apples and in transporting berries to the market. Vigorously growing parts of the plant, *e.g.*, germinating seeds, succumb very quickly in an atmosphere of carbon dioxide with symptoms of autointoxication from products of their vital activity.

34. Influence of Gravity on the Position of Growing Organs. Geotropism.—Independent of the position occupied by the seed in the soil, the seedling always develops its root vertically downward and its stem upward. Thus the root descends into the soil and begins to absorb water and nutrients, while the stem rises to the surface and presents its leaves to the sun's rays, which play the main part in the formation of organic substances by the plant. This orientation of the organs in relation to the force of gravity is retained throughout the life of the plant. The main axis of both stem and root grow vertically, the lateral shoots and roots grow at some definite angle in relation to the main axis, while the leaves are usually adjusted in a more or less horizontal plane depending on the direction of light. If the plant is disturbed from this orientation by some external influence, *e.g.*, if it is bent toward the ground by wind, either the entire plant rises, as may be observed in cereals after "lodging," or the younger parts curve so that the newly developing organs again assume a normal orientation with respect to gravity. This latter form of reaction is found in most plants.

The production of such curvatures leading to a restoration of the normal orientation of plant organs is closely connected with

the phenomenon of growth, and for this reason the curvatures of growing organs have been designated the "growth movements" of plants. There may be distinguished two types of movements: (1) tropisms produced by directive stimuli, *e.g.*, gravity or light; and (2) nasties, curvatures produced by stimuli that affect the whole plant uniformly and do not determine the direction of response, *e.g.*, changes in temperature, humidity of the medium, or the general illumination. Growth movements are the result of the unequal growth of tissues on the different sides of the bending organ. The side that grows faster becomes convex, while the side that is slower in its growth becomes concave; and the organ curves to the side on which growth is slower. Such movements of course may be observed only in organs growing rapidly, or at least in organs that have not stopped growth.

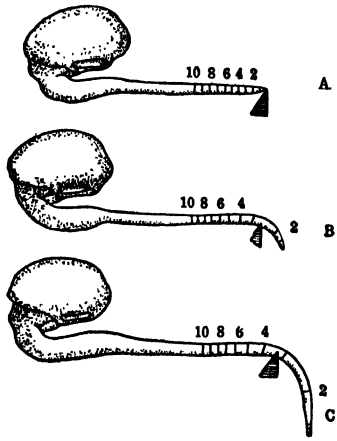


FIG. 37.—Geotropic curvatures of bean roots in humid air. *A*, the original horizontal position; *B*, 7 hr. later; *C*, 24 hr. later. The dark triangle is a paper mark fixed on glass (*after Sachs*).

This relation may be easily observed in the following simple experiment. If seedlings, *e.g.*, of peas or beans, are placed horizontally, after a period of a few hours the stems will turn upward, while the root will bend forcibly downward. If these organs are previously marked at intervals with India ink, as is done for the purpose of studying the rate of growth, it will be noticed that the greatest curvature occurs in the region of the most rapid elongation. The fully grown parts do not manifest any tendency to curve, remaining in the same position in which they were placed (Fig. 37).

A seeming exception to this general rule are the culms of cereals, which are capable of rising after "lodging," by forming a bend just above the node (Fig. 38). This phenomenon, which is of great importance in saving lodged grain, is due to the fact that the stem tissues above the nodes of cereals retain the ability to renew growth for a considerable time. As soon as the stem has been bent into a horizontal position, the lower side of the

node starts growing, thus forming a curvature and raising the part of the stem situated above it.

It is natural to attribute the regularity in the situation of the axial organs of plants to the force that always and everywhere acts in a strictly vertical direction, the force of gravity. The ability to assume a definite position in relation to gravity is called "geotropism." Three types of geotropism are distinguished, *viz.*: positive geotropism of roots, growing downward; negative geotropism of the stems, growing upward; and diageotropism of the leaves, situated horizontally. That gravity is the directing force in geotropism was definitely proved at the beginning of the nineteenth century by Knight, who solved the difficult problem by means of a very clever device. The difficulty of finding sufficient proof in this instance was due to the fact that, because of our existence on the earth's surface, we are unable to escape the effect of the force of gravitation and cannot therefore observe the conduct of a plant, that is not subject to the influence of gravity.



FIG. 38.—The rising of a cereal plant due to renewed growth and a curvature formed by the nodes (*after Nathansohn*).

Not being able to remove entirely the objects of his study from the effect of gravity, Knight nevertheless succeeded in discovering a simple method of avoiding its unilateral effect. He placed the seedlings of various plants on a small wheel, which was rotated on a horizontal axis. This wheel was kept in motion by means of a stream of water obtained from a brook, while the seedlings, being dipped into the water at every rotation of the wheel, were prevented from drying out. With each turn of the wheel, the shoots passed through all the possible positions of orientation to gravity at each revolution. As a result of such continuous rotation, these plants no longer had any continuous

exposure to gravity, its effect being neutralized. The direction of the force of gravity being continuously changed, the shoots grew in any direction that the investigator chose to give them.

This apparatus, called "Knight's wheel," is rotated at rather high speed. This not only helps to prevent the unilateral effect of the force of gravity but also creates a somewhat complicated situation by introducing the effect of centrifugal force, which acts in the same way as gravity. To be able to study better the consequences resulting from the removal of the effect of gravity, Sachs modified somewhat Knight's method by substituting for the water wheel a clock mechanism called a "clinostat." By

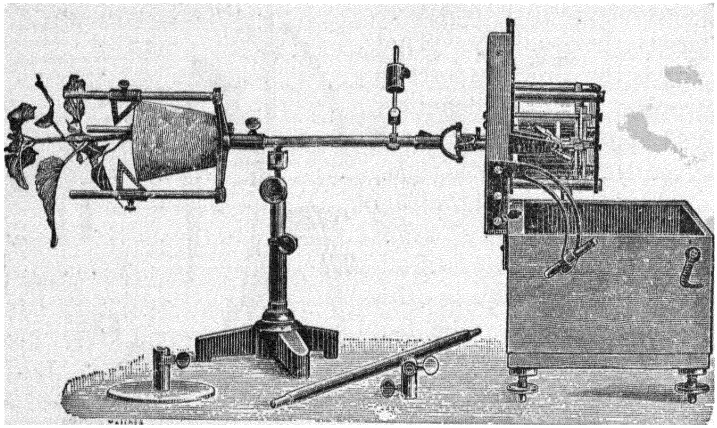


FIG. 39.—A clinostat of Pfeffer's construction (*after Beneckc-Jost*).

means of this device, a rather slow but very regular rotation is attained, without the development of any appreciable centrifugal force (Fig. 39). The essential part of the apparatus consists of a powerful clock mechanism, which is attached to an axial rod. This axis may be adjusted in any desired direction and set in motion at any required speed, most frequently 1 to 10 rotations per hour. This speed has been found to be sufficient to remove the unilateral effect of the force of gravity, so that it has insufficient time to produce the reaction of curvature in a definite direction.

If a seedling is placed in a horizontal position and is rotated slowly on the clinostat, no curvatures will be produced. But if before the clinostat is set in motion, the seedling is left for several minutes in a horizontal position, not so long, however,

as to cause it to bend, then after a certain time it will form a curvature on the clinostat. The original upper side of the stem will become concave, while the original upper side of the root will become convex. In other words, the stem and root will bend in the same way as if they had been left motionless in a horizontal position.

This experiment clearly proves that owing to the unilateral effect of the force of gravity, there is produced in the plant a condition of excitation that is not the same on its upper and lower sides. After a certain period of time, this leads to a definite reaction, the formation of a curvature, in spite of the fact that during this period the cause that produced excitation, the unilateral effect of gravitation, was removed by means of slow rotation. The least duration of stimulation necessary to produce a visible curvature is called the "presentation time." Usually it is very short, amounting in the most sensitive plants, like the stem of the sunflower, to 2 to 3 min., while the time for reaction requires 45 to 60 min. and sometimes even several hours.

The force of gravity is nearly uniform at the earth's surface. By changing the angle of deviation from the vertical line, however, it is possible to modify the intensity of geotropic stimulation, which appears to be in proportion to the sine of the angle of deviation from the vertical. This is known as the "law of the sine." Therefore, the time of presentation seems to be least in a horizontal position and increases with the gradual approach to the vertical line. The product obtained by multiplying the time of presentation by the sine of the angle of deviation is a constant quantity.

This constant indicates that the realization of the geotropical reaction requires a definite amount of stimulus, there being no difference between the prolonged action of a weak stimulus and action for a short time of a strong stimulus. This relation has been summarized as the law of the quantity of stimulus. This law holds good not only for the phenomenon of geotropism, but likewise for phototropism and other movements of the plant, as well as for the phenomena of excitation in the animal organism.

By changing the angle of deviation, it is possible only to decrease the strength of the geotropic stimulus. In order to increase it, it is necessary to apply the effect of a different force, which also produces an acceleration of mass, *viz.*, centrifugal

force. If the shoots of plants are fixed in a vertical position on the circumference of a wheel that is rotated horizontally at considerable speed, it will be found that during further growth, the seedlings will deviate from their vertical position. Their stems will be directed toward the center, and the roots away from it. By altering the speed of rotation, it is possible to change the angle of deviation from the vertical line. By this means, it has been found that the direction of the axial organs will always correspond to the resultant between the force of gravity and centrifugal force. In case of very rapid rotation, when the centrifugal force is greater than that of gravitation, the shoots will grow almost horizontally. And when the speed of rotation is such that the centrifugal force is equal to the force of gravity, then the shoots will grow at an angle of 45 deg.

By subjecting the shoots to the effect of centrifugal force during a short period and then transferring them to the clinostat, it is possible to obtain the same curvatures as are secured after a short exposure to the effect of gravity. It is also possible to determine definitely the presentation time for each intensity of centrifugal force. This time has been found to be in inverse proportion to the magnitude of the force used. In this case, also, one secures a full confirmation of the law of the quantity of stimulus.

Geotropic stimuli are not equally perceived by all parts of an organ possessing geotropic sensitivity. The distribution of geotropic sensitivity has been most studied in the root. Charles Darwin (1860) showed that the tip of the root, or the terminal 1 to 2 mm., is the region of sensitivity and that roots with the tips cut off grow horizontally, having lost the capacity to perceive the direction of gravity. In seedlings of grain, the geotropic sensitivity is localized in the tip of the coleoptile; while in the majority of dicot stems, it is more or less equally distributed throughout the growing zone.

The question as to exactly how plants perceive the direction of gravity is of great interest but can by no means be regarded as finally solved.

The mechanism of this perception remained for a long time unknown. Only the recent development of experiments on growth hormones opened the possibility of a significant advance in the solution of this difficult problem. The coincidence of the

zone of geotropical sensitivity of different organs with the zone that produces growth hormones served as a point of attack for the further solution of the question.

Experiments by Cholodny (1926) have shown that decapitated roots regain their lost geotropic sensitivity if tips of roots or even tops of the coleoptile of corn are placed on the surfaces of the cut. Similarly, a section of the hypocotyl of lupine that owing to the removal of its conductive tissue has lost its geotropic sensitivity (see Art. 29) regains it if supplied with the growth-promoting hormone from the coleoptile. Hence it is evident that the main factor influencing curvature is the presence and distribution of the growth hormone. In the case of a horizontally placed organ, a unilateral distribution of the hormone flow may be observed, *viz.*, in the lower side, which according to Browner (1927) is negatively charged. Thus the cause of the deviation in the flow of the hormone is probably the electrical polarization of the cells.

The very important and interesting question as to why roots react differently from stems to the force of gravity, in spite of the fact that both produce the same hormone, is explained by Cholodny on the basis of the different action of hormones on the roots and stems. Under the action of the growth hormone, the rate of growth of stems is increased, while that of the roots is decreased. This is shown also by the fact that decapitated stems grow more slowly than uninjured ones, while decapitated roots grow faster than normal ones. Moreover, experiments have shown that one and the same hormone, *e.g.*, the hormone secreted by the tips of the coleoptile of corn, retards the growth of positively geotropic organs and increases that of negatively geotropic organs. Although the growth hormone in horizontally placed stems and roots flows mainly to the lower side, the result of its action is different; the accumulation of the hormone in stems increases the growth of the lower side and causes the organ to bend upward, while in the case of roots, the growth of the lower side is decreased and the bending is downward. Consequently, the difference in the geotropic reaction can be explained by a dissimilar reaction of the cells of roots and stems to the hormone that diffuses into them (see Art. 30).

Geotropic sensitivity is not quite constant and may be influenced by external as well as by internal conditions. Owing to

the effect of low temperatures, negative geotropism of stems, for instance, may change to transverse geotropism, and then the shoot begins to grow in a horizontal position. This may be observed in many spring plants, which cling to the soil during the early cold period and only later in the season begin to grow upward. Similar changes in geotropic behavior may be seen also when plants are subjected to the effect of certain gases, ethylene and acetylene in particular. Plants appear to be extremely sensitive to these gases. According to Neljubov's investigations, 0.002 cc. of ethylene per liter of air is sufficient to cause the seedlings of peas or vetch to assume horizontal growth. In laboratories supplied with gas, it is therefore impossible to obtain normal seedlings of such sensitive plants.

Similar changes in geotropic behavior are produced also by internal causes. Many rhizomes grow horizontally throughout the summer but produce a vertical shoot during the following spring. The peduncle of poppy exhibits positive geotropism before the period of flowering, and therefore its tip is directed downward. This is by no means caused by the weight of the bud, as it was formerly supposed, for to straighten the peduncle an amount of force far in excess of the weight of the bud has to be applied. Toward the period of blooming, the geotropic "behavior" of the peduncle is changed, and it straightens out, owing to the effect of the newly developed negative geotropism. The causes that produce these changes in geotropic behavior are not understood at present.

35. Influence of Light upon the Direction of Growth of Organs. Phototropism.—Light is one of the factors indispensable to the life of green plants, providing them with the energy required for the decomposition of carbon dioxide. It is but natural therefore that plants should display a high degree of sensitivity in relation to light and should respond effectively to changes in the direction and intensity of the light rays falling upon them. The ability to react to the directive influence of light is called "phototropism." Young growing stems usually curve toward the source of light, such a reaction being termed "positive phototropism" (Fig. 40). Curvature in the direction opposite to incidence of light is "negative phototropism," while the capacity of adjusting organs, *e.g.*, leaf blades, in a direction perpendicular to the incident rays is known as "diaphototropism." As a rule, dorsi-

ventral organs such as leaves or the thallus of the liverworts are diaphototropic, while axial organs of a radial structure generally exhibit either positive or negative phototropism.

Not all the organs of a plant are phototropically sensitive. This is a characteristic feature only of the aerial parts of plants. The underground organs, such as roots and rhizomes, which normally grow in darkness, very often do not react to light. Some roots, however, like those of mustard and other crucifers, are negatively phototropic.

The capacity of bending toward or away from light is not permanent in every organ. It depends on the intensity of light.

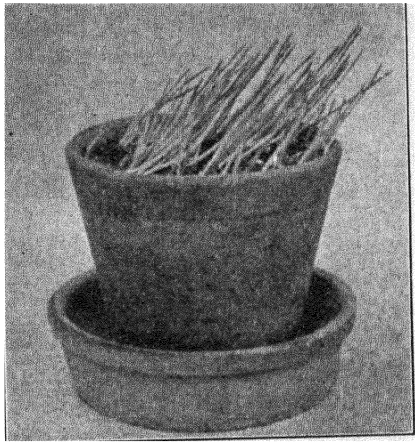


FIG. 40.—Seedlings of oats bending toward light (*after Nathansohn*).

In too strong light, positive curvatures frequently change to negative ones. By selecting various intensities of light, it is possible, therefore, to make the same shoot bend in succession toward and then away from the source of light. It is also possible to find a certain intensity of light in which the positive effect will be neutralized by the negative; the plant will then appear to be insensitive to light.

Phototropism plays an important part in the life of plants. On account of it, leaves, which require light, assume the position that is most favorable for the utilization of this source of energy. They usually spread perpendicular to the direction of the incident rays (Fig. 41). Owing to phototropic movements, the leaves of many plants form the so-called "leaf mosaic," which is

particularly conspicuous in plants growing in shady places, *e.g.*, the ivy (Fig. 42). In general, phototropic phenomena are more distinct in plants growing indoors or in the shade than in those grown in open spaces. This is owing partly to the fact that in very strong light phototropic sensitivity is somewhat lowered. It may be caused also by the diurnal and continuous change in the direction of sunlight. Some plants, however, show so rapid

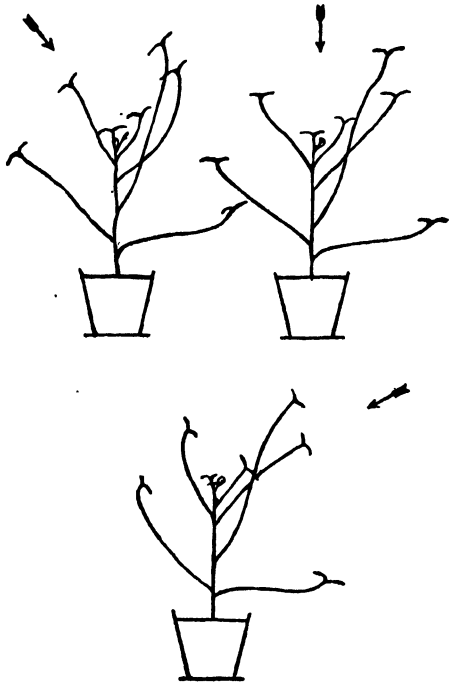


FIG. 41.—A geranium adjusting its leaves perpendicular to the direction of light (*after Lubimenko*).

a reaction to an alteration in the direction of light that they follow the motion of the sun. Good examples of such types are the sunflower, *Helianthus*, and species of *Bidens*.

Phototropic sensitivity is in no way connected with the presence of chlorophyll. Sporangia and the fruiting bodies of many colorless fungi also manifest phototropic curvatures (Fig. 43). Moreover, etiolated seedlings are always more sensitive to light than green ones. In general, it has been found that the growing of plants in light decreases their phototropic sensitivity

in proportion to the intensity of light; therefore, plants in open spaces are less sensitive to light than those growing in the shade, while the most sensitive are those that have grown in total darkness. The latter are, as a rule, used for precise phototropic experiments. These experiments are carried out in the photo-

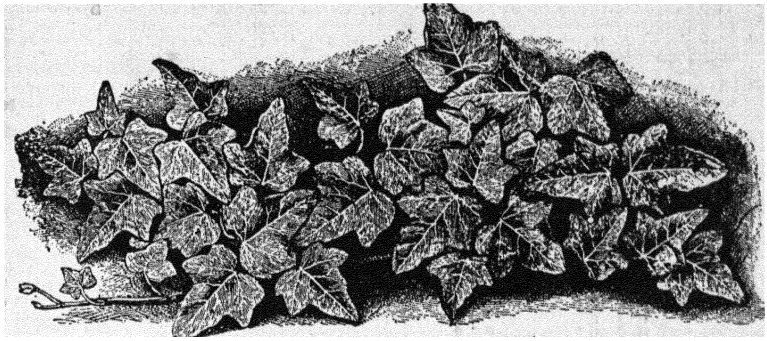


FIG. 42.—The leaf mosaic of an ivy (*after Lubimenko*).

tropic chamber, a tight box having the interior painted black and for experimental purposes arranged to admit light through a small opening in one of the walls (Fig. 43).

The seedlings of some cereals, particularly those of sorghum and millet (*Setaria*), are most convenient objects for such experiments. These seedlings consist of a rather long hypocotyl bearing a comparatively small coleoptile, which encloses the first true leaf. If the shoot is placed in a phototropic chamber, the hypocotyl will form a distinct curve, and the tip of the coleoptile will be directed toward the light (Fig. 44). At first the curvature is formed directly beneath the tip of the coleoptile, but later, as further growth of the hypocotyl takes place, the curvature is in a somewhat lower region. By means of simple experiments, it may be easily shown that the coleoptile is the organ that perceives light, while the hypocotyl merely responds to the stimulus transmitted from the coleoptile. If an opaque hood

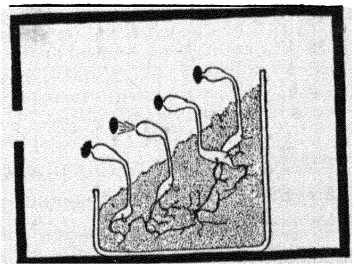


FIG. 43.—Sporangia of *Pilobolus* turned toward the openings of a phototropic camera (*after Smith et al.*).

made of black paper or tin foil is placed over the tips of the shoots so as to cover the whole of the coleoptile, no curvatures are formed, notwithstanding the fact that the hypocotyl remains exposed to light. On the other hand, if the hypocotyl is shaded from light and only the coleoptile remains exposed to it, curvatures are formed as distinctly as when the whole of the shoot is exposed.

The mechanism of phototropic curvatures consists in a more retarded growth of the exposed side of the stem as compared with the growth of the shaded side; thus the lighted side becomes shorter, and the entire organ will curve. Comparing this fact with the long-known phenomenon of delayed growth caused by the effect of light, many authors, beginning with De Candolle, attributed this bending of plants toward light to the direct retarding effect of light on the growth of the exposed side. But considering the experiments that have demonstrated that perception is localized in the tip of the shoot, which is not directly subject to curving, the foregoing explanation has to be modified as follows: A unilateral exposure to light causes certain substances to appear in the organ of perception; these descend to the growing zone and produce a different effect upon the growth of the exposed and the shaded sides.

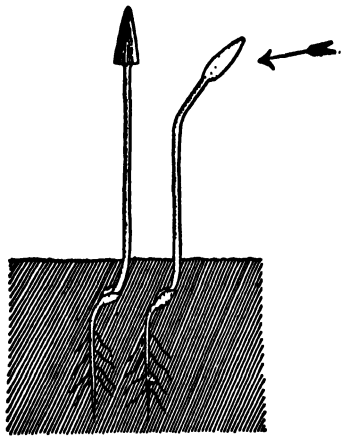


FIG. 44.—Seedlings of *Setaria*. On the right, one bent toward light; on the left, one of the seedlings has remained upright. Its plumule has been covered with a non-transparent cap (*after Holman and Robbins*).

If all the data concerning the physical nature and the manner of the transmission of phototropic stimuli are correlated with the dependence of the rate of growth (Art. 29) on the amount of the growth hormone manufactured by the stem tip or the coleoptile, the following mechanism of phototropic curvatures may be observed (Cholodny, Went). Under the influence of the stimulus of light, the total amount of the growth hormone in the sensitive apex, as well as its distribution, is altered. As the exact quantitative determinations by Went have shown, light

in itself decreases the amount of the growth-promoting substance in the apex. This explains the retarding influence of light on growth. Moreover, under the influence of unilateral illumination, there takes place an electrical polarization of the cells that produce and transmit the growth-promoting substance. The illuminated side receives a negative, the shaded side a positive, charge. Under the influence of this polarization, the current of growth-promoting substance is shifted to the shaded side. The cells of this side elongate more rapidly and more strongly than do those of the opposite side, and as a result a curvature of the seedling toward the light appears.

Not all the rays of the solar spectrum produce an equal phototropic effect. Red rays, as a rule, are least effective. Toward the blue end of the spectrum, there is an increased effect, which attains its maximum in the indigo-blue rays (4650 Å.) and then again gradually decreases toward the ultraviolet region.

36. Chemo-, Hydro-, and Thermotropism.—Besides the force of gravity and light, there are a number of other factors that may cause curvatures in the growing organs of plants, if they are subjected to a unilateral exposure. These tropisms, however, have been studied very little. Moreover, their role in the life of a plant is comparatively small. Therefore, discussion of them will be limited to only a short review.

Chemotropic curvatures are caused by an unequal distribution of certain chemical substances in the environment of a plant. Chemotropism is of the greatest importance in the life of fungi and other saprophytic and parasitic plants. It directs the growth of their hyphae and haustoria toward the source of nutritive substances. There are two kinds of chemotropisms, positive and negative. The former is caused chiefly by various nutritive substances, *viz.*, sugar, peptone, asparagine, phosphates, and ammonia compounds. Negative chemotropism manifests itself when acids and alkalies or salts of heavy metals are applied. Chemotropism may be produced also by certain salts, such as potassium nitrate and magnesium sulphate.

A good method for the study of chemotropism in fungi is to sow their spores on a perforated plate of mica, placing it over a layer of gelatin that has been previously soaked in the substance that is being investigated. In the case of positive chemotropism, the fungus hyphae will penetrate through the perforations; but

when negative chemotropism is exhibited, they will draw away from the openings. The Japanese physiologist Miyoshi has used leaves, previously injected with various solutions, for the same purpose. With this method, positive chemotropism caused the hyphae to penetrate through the stomata into the inner tissues of the leaf, a picture very similar to that observed in cases when the leaf is infected by certain parasitic fungi.

The specific effect of various substances will depend upon their concentration. Excessive concentrations may change positive chemotropism to negative. Fungal hyphae may exhibit a strikingly high sensitivity to some substances. Thus *Mucor* will show distinct curvatures when subjected to the effect of a sugar solution of as low a concentration as 0.01 per cent.

The growth of pollen tubes through the style to the ovule presents a picture of chemotropism similar to that of fungal hyphae. They invariably find their way to the embryo sac, reacting doubtless, to the substances secreted by the ovule. If an ovule is placed in a drop of water with some pollen in it, the pollen tubes will grow toward the ovarian tissues.

The chemotropism of roots has not been studied sufficiently. The root responses to chemicals present considerable difficulties, as the same substance diffusing into the environment of the root, may produce a double effect on it. It may have a direct influence on the growth rate of that part of the root that comes in contact with the substance, and it may produce also an irritation in the highly sensitive root tip. The two effects may be antagonistic to each other. A toxic substance affecting the side of the root with which it comes in contact produces a local inhibition of growth and, consequently, a positive curvature. The same substance affecting the tip of the root may produce therein a condition of stimulation, which may be transmitted to the growing zone and may result in the formation of a curvature in the opposite direction. It has been possible to separate these two effects by means of a unique method of procedure suggested by Porodko (1911). He placed the seedlings of various plants in a layer of gelatin gel, in which the diffusion of a substance producing a chemotropic effect was known to be free from convection currents. In some of the experiments, the tip of the root was left outside the gelatin; in others, the growing zone was outside. These experiments have led Porodko to conclude that

nonelectrolytes do not produce any chemotropic effect on roots, while the cations of electrolytes, as a rule, produce a negative chemotropic curvature, and the anions a positive curvature, the effect of salts being in this respect made up of the sum of the effects of their cations and anions.

Under natural conditions, the role of chemotropism in regard to the growth of roots is of little importance, at least as far as the effect of solutions in the soil is concerned. The distribution of air and water in the soil appears to have a much greater effect on the growth of roots.

If a vessel containing the root system of a plant is hermetically sealed except for one small opening, then the roots will grow in the direction of this opening to meet the oxygen diffusing inward. This phenomenon has been given the name "aerotropism," it being but a special type of chemotropism. Humidity produces a still greater effect on the growth of roots. When water is not equally distributed through the soil, the roots may grow in the direction of the more humid portion. The bending of roots in the air toward more humid surfaces may be observed (Fig. 45). Such an experiment, however, will be successful only in an atmosphere that is almost saturated with water vapor, for otherwise the roots are apt to dry out. But no hydrotropic curvatures will be secured in an atmosphere completely saturated with water vapor, as in this instance the whole space will be of equal humidity. Like geotropism, hydrotropic sensitivity is localized in the tip of the root.

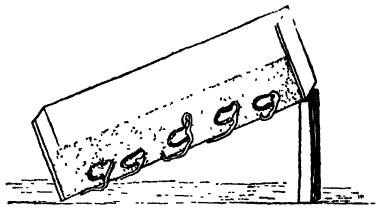


FIG. 45.—Hydrotropism of roots. Instead of growing straight downward, the roots cling to the damp sawdust (*after Osterhout*).

An unequal distribution of temperature may also produce bending of both the roots and the organs above ground, showing both positive and negative thermotropism. When the temperature is below the optimum, plants will curve in the direction of the more heated side; when above the optimum, they will bend toward the colder side. Such thermotropic curvatures may be secured with roots growing in moist sawdust in a box whose ends are maintained at different temperatures.

The study of the reaction of plants to strong chemical and thermal stimulation is to a considerable extent complicated by the phenomenon of traumatropism, which was discovered by Darwin (1881). If the tip of a root is severely injured by means of a unilateral incision or cauterization with an acid, etc., the irritation produced is transmitted to the zone of elongation and produces a negative curvature. Traumatropic sensitivity permits the root to avoid obstacles that may stand in its way. It is, therefore, of ecological importance. The organs above ground, particularly those of seedlings, also exhibit traumatropic bending, but these are chiefly of a positive nature.

Autotropism is the tendency of a plant to grow in its normal form. Thus, in its growth, the stem tip may nutate from side to side depending upon unequal growth on different sides, or it may bend in response to stimuli. Curvatures formed owing to the transient effect of some external stimulus are again straightened out, provided that they have not been definitely fixed by growth. Autotropism also serves to straighten the small curvatures of a casual nature.

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CHAPTER V

ASSIMILATION OF CARBON BY PLANTS

37. General Concept of Photosynthesis. Products of Assimilation of Carbon. Connection with the Assimilation of the Elements of Water.—At germination, the rapidly developing seedling draws upon the reserves stored in the cotyledons or in the endosperm for the nutrients indispensable for its growth. A considerable part of these reserves is expended in the process of respiration to provide the energy necessary for growth. As has already been emphasized, the beginning of germination is therefore a time of rapid loss of dry weight of the seedling. If germination is carried on in darkness for too long a time as when seeds are sown deeply, exhaustion may result in the death of the seedlings.

With the emergence of the first leaflets above the surface of the soil, an abrupt alteration occurs in the character of the nutrition of the young plant. The leaves that were yellow underground become rapidly green under the influence of light and start energetic photosynthesis of organic substances. The expenditure of food reserves, characteristic of the first stage of germination is replaced by their accumulation, and the seedling, which first was nourished at the expense of its reserves, now changes into a plant independently procuring its nutrients from the surrounding medium.

In discussing the elementary composition of the plant, it has already been noted that almost half of the dry substance is made up of carbon. But the significance of carbon is not limited to its quantitative prevalence only. Atoms of carbon, as is known, form the basic skeleton of all organic compounds. The capacity of carbon to give various combinations with other elements explains the enormous diversity of these compounds. When the plant begins its independent existence, the first and most important problem is the absorption of carbon dioxide from the surrounding medium and its synthesis into complex substances to form the structural part of all organs. The primary

and most important role in the life of the plant therefore belongs to carbohydrates.

This process of the building of carbohydrates from inorganic substances of the surrounding medium has been designated "photosynthesis," for it proceeds only with the absorption of light, which supplies the necessary energy.

The initial substances for photosynthesis are carbon dioxide and water, and the whole process may be summarized in the following simplified reaction:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

This reaction shows that during the process of photosynthesis the plant liberates free oxygen into the surrounding atmosphere For the animal world, this oxygen is of major importance, for it serves as a source of renewal of the oxygen in the atmosphere, making it fit for respiration, in spite of the continuous consumption of oxygen by animals and nongreen plants, especially by bacteria and fungi and in combustion processes. The liberation of oxygen by plants has played a great part in the history of the process of photosynthesis. It was first observed by Priestley (1772) in aquatic plants, which are especially convenient for such observations; oxygen, being little soluble in water, appears in the form of bubbles easily detected in sunlight. The capacity of plants to "improve" the air was fully appreciated by early investigators long before the significance of photosynthesis for the nutrition of plants was recognized.

The simplified reaction of photosynthesis, given above, shows that the process of assimilation of carbon is simultaneous with the assimilation of the elements of water and that its essential products are carbohydrates. In fact, it is not difficult to prove by means of direct analyses that the carbohydrate content of leaves increases considerably during the daylight and diminishes significantly at night when, owing to the absence of light, the formation of carbohydrates is checked, while their consumption through respiration and their outflow into other parts of the plant continue. Since by photosynthesis about 90 per cent of the total dry substance of the plant is formed, very often the whole process has been called "assimilation." This term is fully justified, since assimilation of carbon, hydrogen, and oxygen is closely connected with the assimilation or transformation into

organic compounds of the other elements such as nitrogen, sulphur, phosphorus, etc., that enter into the composition of the protoplasm.

Of all the carbohydrates synthesized in the plant, it is most easy and convenient to observe the formation of starch, for it accumulates in grains that may be seen under the microscope. Starch may be detected also by the well-known iodine reaction. By staining with IKI solution, the formation of starch grains in the green plastids of plants may be easily followed under the microscope especially in the thin leaves of aquatic plants (Fig. 46), in mosses, and in the cells of algae. With sufficient illumination, this process will take place under the microscope, and the increase in size of the starch grains in the chloroplasts may be seen directly. In direct sunlight, 5 min. is sufficient for the formation of starch. Even with the comparatively weak light of a petroleum lamp of 100 foot-candle power, Famintzin as early as 1860 observed the formation of starch in the cells of *Spirogyra* in 30 min. after the beginning of the experiment. Another convenient method for determining the accumulation of starch is the well-known Sachs test. A leaf, having been previously exposed to light, is decolorized by means of alcohol and afterward treated with a solution of iodine dissolved in potassium iodide. If separate areas of the leaf are shaded with opaque paper prior to the experiment, there will be produced light figures on the general iodine-stained background of the leaf. The relationship between the amount of light transmitted and the accumulation of starch is so close that by means of this method it is possible even to print in the leaves portraits from negatives just as on photoprint paper (Fig. 47).

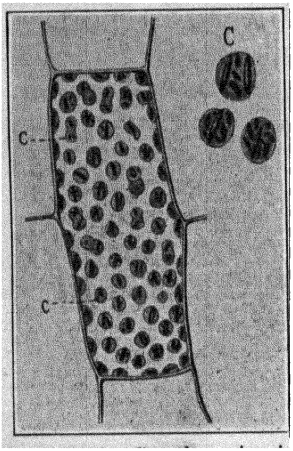


FIG. 46.—Starch grains in the chloroplasts of Elodea (*after Molisch*).

Starch is the most easily detectable product of assimilation. This does not mean, of course, that it is the first product, for it represents a far too complex structure made up of large molecules. Its production in the leaf is preceded by the formation

of different kinds of sugars. Exact quantitative determinations show that usually not over 30 to 50 per cent of the products of assimilation are deposited in the form of starch, the rest being sugars and other compounds. Still less starch is formed in the leaves of wheat. In many plants, *e.g.*, the onion, tulip, *Arum*, etc., the process of assimilation terminates with the formation of sugars. Such plants are called "sugarformers" in distinction to the usual starch formers. The accumulation of starch must be regarded as a secondary process, the purpose of which is to withdraw as soon as possible the soluble sugars from the sphere of the primary assimilative reaction, since the accumulation of

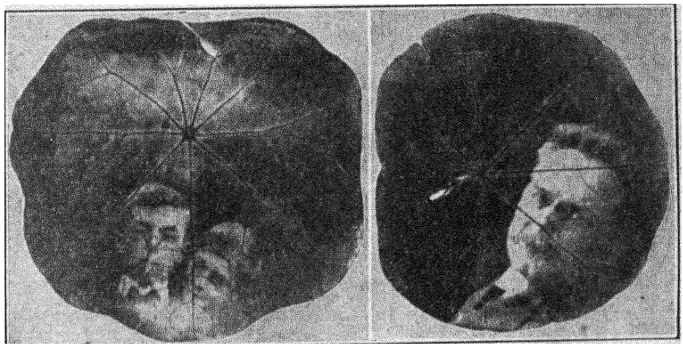


FIG. 47.—Prints from negatives on leaves made by means of a starch test (*from Molisch*).

soluble end products always checks the progress of the reaction (Sapozhnikov effect). The accumulation of starch has no direct connection with the process of carbon assimilation. Experiments have shown that it may go on in leaves at the expense of sugar obtained from without, *e.g.*, when leaves are placed in a sugar solution and kept in a dark room.

The accretion of organic substance, sugars or starch, in the process of assimilation represents but one side of this most important process. These substances may be burned with liberation of heat energy, as in the case of burning wood or coal. When vegetable products are used for food, they are oxidized within the body through respiration, and energy is obtained from them, which is used in maintaining the temperature of the body at a certain level (about 37°C.), as well as in work performed. Assimilation is not only a process for the accumulation of organic

matter but also a means of storing energy in the form of a combination of carbon and hydrogen capable of being oxidized. This potential energy is accumulated from the radiant energy falling on the leaf, which is caught by the chlorophyll and transformed into chemical energy. The thermodynamics of the assimilation process, which plays such an important role in the general energy exchange in the world, will be discussed in detail in Art. 41. The potential energy accumulated by the assimilation process can be gauged by the immense reserves deposited in the form of peat, coal, and mineral oil, which represent the chief motive power of the world's industry.

38. Methods of Detection and Study of Photosynthesis. At present there are numerous methods by means of which it is possible not only to detect the process of assimilation of carbon dioxide by the plant but also to study this process quantitatively, as the following discussion will indicate.

To determine the amount of carbon dioxide that is assimilated by the plant, an air current in which the amount of carbon dioxide has been determined beforehand is passed through a jar containing the plant. The unused portion of carbon dioxide is then absorbed by an alkali. This method shows that in bright light leaves are able to absorb almost all of the carbon dioxide from the passing air if the current does not move too fast. Boussingault was the first to employ this method about a hundred years ago. In recent times, it has been considerably improved by other investigators. This method has been used widely in studies by Kostytchev, Lundegårdh, Boysen Jensen, and others. Of the many types of absorbers, most simple and convenient is the one designed by Ordojan. It consists of a glass tube about 50 cm. in length filled with barium hydroxide solution. At the base of this tube is a finely perforated metal sieve, which breaks the air current into very fine bubbles, thus facilitating more complete absorption of carbon dioxide. The part of the plant to be investigated is placed in a special glass chamber, through which the air current is drawn rapidly by means of an aspirator. The air then enters into the absorber through a lateral glass tube connected with the absorber at its base, and the carbon dioxide is absorbed by the barium hydroxide solution.

Simultaneously, by means of a similar aspirator and absorber, determinations are made of the carbon dioxide contained in the

same amount of air as that drawn through the glass chamber. The difference between the quantity of carbon dioxide contained in the air directly entering the absorber and that of the air previously drawn through the chamber with the plant gives the quantity of carbon dioxide consumed by the latter. The leaves or branches may be placed in the chamber without being detached from the plant, and thus the experiment may be performed under "field conditions." This method has the advantage of permitting the study of the absorption of carbon dioxide in natural surroundings or in constant atmospheric conditions. A defect of this method is the overheating of the glass chamber that always occurs when the experiments are carried out in direct sunlight.

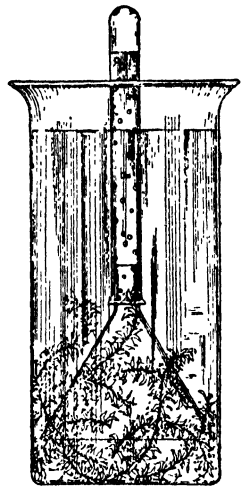


FIG. 48.—Method of gathering oxygen given off by an aquatic plant (*after Palladin*).

A number of methods of study of carbon assimilation are based on the principle that in photosynthesis the absorption by the plant of carbon dioxide from the atmosphere is accompanied by the evolution of an equivalent amount of oxygen.

There are several means of detecting the liberation of oxygen. The simplest procedure is to use plants that are submerged in water to which a certain amount of carbon dioxide has been added. When such plants are exposed to a sufficiently bright light, *e.g.*, direct sunlight or a powerful electric light, gas bubbles will appear on the surface of the plant. When these bubbles are gathered by means of a funnel into a test tube or eudiometer, it is easy to prove that the gas is oxygen by introducing a glowing splinter, which with a high concentration of oxygen bursts into flame. This experiment is most easily conducted with water plants (Fig. 48). It may be demonstrated with a branch of *Elodea canadensis* with the cut end turned upward. When placed in the light, air bubbles will rise with great regularity, issuing from the cut end. In counting the number of bubbles that are given off during 1 min., changes in the rate of carbon dioxide assimilation may be observed. This method is not very exact, since by diffusion, other gases such as nitrogen and carbon

dioxide may be dissolved in water and will mix with the oxygen released. The method most commonly applied in recent years is an exact quantitative determination of the oxygen evolved by the plant, by means of titrating a definite volume of water with oxygen-absorbing substances (Winkler's method).

The most complete idea of the process of assimilation of carbon dioxide is obtained with procedures by which not only the amount of carbon dioxide absorbed is determined, but that of the oxygen released as well. The method most commonly used is the one in which the leaf of a plant is placed in a eudiometer sealed with mercury and filled with air of a definite carbon dioxide content (Fig. 49). The eudiometer is then exposed to light; and after some time, analysis is made of the gas inside. By comparing the amount of carbon dioxide consumed with the amount of oxygen accumulated in the eudiometer, information is obtained about the relative changes that take place in the composition of the air resulting from the activity of the plant.



FIG. 49.—Eudiometer for the study of photosynthesis (*after Kostytchev*).

Methods of analysis of the gas contained in the eudiometer have already been described in the discussion of the methods for the study of respiration.

In recent years, photosynthesis has frequently been determined by means of the Warburg manometric method described previously in Chap. III. The change in the total volume of gas during photosynthesis is caused by the fact that oxygen is considerably less soluble in water than is carbon dioxide.

A somewhat indirect, but nevertheless very important, method of studying the assimilation of carbon is the so-called "dry-weight method" devised by Sachs. This determination is based on the fact that as a result of the accumulation of the products of assimilation in the leaf blade, it becomes heavier, and this increase in weight may be ascertained by means of accurate analytical balances. It is necessary, of course, to weigh not a fresh but a dried leaf, as in a recently cut leaf the fluctuation in the water content overbalances variations in the amount of dry matter. Because a dry leaf does not assimilate, it is necessary to resort to

a somewhat roundabout way to ascertain the added weight of dry matter. A leaf showing as far as possible a symmetric structure is taken for the experiment. One-half of the blade is cut away along the midrib. From this half, a portion of a precisely defined area is cut out, dried, and weighed. By dividing the weight by the area, the initial dry weight of a surface unit of the leaf is obtained. The other half of the leaf blade, remaining in connection with the midrib, is exposed to light. After several hours, the weight of a surface unit is determined in the same way. The increased weight in comparison with the first half gives the increase in dry matter per surface unit.

The determinations made by Sachs, according to this method, have shown that a sunflower leaf in bright light accumulates in 1 hr., 0.914 g. of dry matter per square meter; a pumpkin leaf accumulates 0.680 g.; a leaf of rhubarb, 0.652 g. The exact values of assimilation, however, must be considerably higher, as two other processes leading to the loss of dry matter are going on simultaneously in the leaf. These processes are the translocation of the substances that are produced in assimilation, from the leaf to the stem, and respiration, which is a continuous oxidation of organic matter. If this loss of substance during the experiment is taken into account, by placing in the dark a second leaf similar to the first one and then determining the loss in dry matter per surface unit, the values of assimilation will be considerably higher than those given above. Sachs has determined the total dry weight of the organic matter formed in 1 hr. per square meter as 1.7 to 1.9 g. for the sunflower leaf and 1.5 g. for the pumpkin leaf. These experiments have been repeated by other investigators with similar results. Hence, it may be accepted, as average values at least, that during 1 hr. of a summer day the plant assimilates 1 g. of dry matter per square meter of its surface.

Besides these principal methods of studying the absorption of carbon dioxide by the plant, there are other methods of a more special character. Some of these will be mentioned later.

39. Green Plastids as Special Organs of Photosynthesis. Chlorophyll, Its Chemical Composition, Structure, and Properties. Pigments Accompanying Chlorophyll.—The assimilation of carbon dioxide takes place in special cell structures, the green plastids. An indirect proof of this is the fact that only the green

parts of the plant can absorb carbon dioxide and liberate oxygen. A direct proof was obtained by Engelmann by means of a very sensitive bacterial method for detecting oxygen. Many bacteria show mobility only in the presence of oxygen, even in small quantities, and will move toward the source of oxygen. If a unicellular alga is placed in a drop of liquid containing such bacteria and the preparation is exposed to light, all the bacteria will gather around the alga as a source of oxygen. Care must be taken, however, to coat the edges of the cover glass with vaseline to prevent the diffusion of oxygen from the surrounding air. If instead of a single-celled alga, a thread of *Spirogyra* is studied, the chloroplast of which has the form of a spiral band with large portions of hyaline protoplasm between the coils of this spiral, an accumulation of bacteria will be observed near such parts only of the cell wall as are contiguous to the spiral chloroplast. The movements of bacteria will be observed near the chloroplasts that have been separated from destroyed cells, though, in this case, the activity of the chloroplasts continues but a short time, after which they die. Chloroplasts consist of a protein body, called the "stroma," impregnated with several pigments, among which may be mentioned two green pigments of chlorophyll, chlorophyll *a* and chlorophyll *b*, and two yellow pigments, carotene and xanthophyll.

For assimilation, the harmonious and concordant functioning of both stroma and chlorophyll is absolutely indispensable. This is obvious from the fact that it has never been possible to reproduce the decomposition of carbon dioxide in solutions of chlorophyll in various kinds of liquids, alcohol, benzine, oil, etc. On the other hand, the colorless plastids (leucoplasts) as well as the yellow pigments are not capable of photosynthesis.

The importance of chlorophyll in the process of carbon assimilation will be discussed further on. It may be stated here that it renders the energy of the absorbed light effective in the decomposition of carbon dioxide. In all probability, it takes part in this process chemically also, entering into combination with carbon dioxide and making it accessible to the action of light. The primary importance of the stroma consists in forming the structure through which the particles of chlorophyll are distributed and which, in a way not yet understood, makes their functioning possible. It is also the carrier of enzymes that

decompose the first unstable products of the combination of chlorophyll with carbon dioxide, thereby promoting their further transformation into carbohydrates. The stroma has still another important function: it contains enzymes for the synthesis of starch from the sugar that originates in the process of photosynthesis. This function is possessed not only by the green but also by the colorless plastids, *e.g.*, the starch-forming leucoplasts in the cells of potato tubers and other underground storage organs. The only difference between the processes of starch synthesis in chloroplasts and leucoplasts is that in the stroma of green plastids the starch is formed from sugar found in them, while in the colorless leucoplasts the material that serves for the synthesis of starch is the sugar translocated from the leaves. The process of starch formation in both plastids must be regarded as secondary, not connected directly with photosynthesis.

Chlorophyll is one of the most interesting of substances, for its presence makes possible the highly important synthesis of an organic compound from the inorganic CO_2 and H_2O. It is the only pigment capable of doing this in higher plants. At the same time, chlorophyll is one of the most complex of organic substances. Its structure and composition have been ascertained only recently after the efforts of a number of eminent scientists, covering a period of over 100 years. Among these, the classical works of Willstätter (1910 to 1915) and his coworkers are outstanding. The difficulty of studying chlorophyll depends chiefly on its quick decomposition, requiring special treatment with neutral solvents to extract it unchanged from the plant. Ethyl and methyl alcohol and acetone are the most common reagents used for extraction, after which the chlorophyll is transferred to benzine or petroleum ether to be purified.

According to the investigations of Willstätter, the purest chlorophyll, freed from the admixture of yellow pigments, is composed of two very similar substances, called "chlorophyll *a*" and "chlorophyll *b*." The chemical composition of chlorophyll may be expressed by the following formulas:

Chlorophyll *a*	$C_{55}H_{72}O_5N_4Mg$
Chlorophyll *b*	$C_{55}H_{70}O_6N_4Mg$

Evidently the difference between the two kinds of chlorophylls is that *b* has 2 atoms of hydrogen less and 1 atom of oxygen more

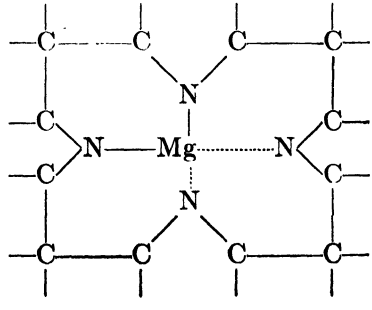
than *a*. The transformation of one into the other, however, may be accomplished neither by oxidation nor by reduction, the difference between them thus proving to be more complex than simple oxidation or reduction. They differ somewhat also in their color, chlorophyll *a* being of a bluish-green, and chlorophyll *b* of a yellowish-green tinge. The amount of chlorophyll *a* is usually greater than that of chlorophyll *b*. In general in higher plants, for every 3 molecules of chlorophyll *a* there is present 1 molecule of chlorophyll *b*. This ratio varies somewhat in different plants. The total amount of chlorophyll in a plant is not great, constituting, on an average, only about 1 per cent of the dry weight. To secure such quantities of chlorophyll as are required for exact chemical investigations, great quantities of plant material have to be used.

Chlorophyll may be considered a complex ester of a dicarboxylic acid, chlorophyllin, in one carboxyl of which hydrogen is replaced by the methyl radical, in the other, by the radical of an unsaturated monatomic alcohol with a rather long carbon chain, called "phytol," and of the formula $C_{20}H_{39}OH$. The more detailed formula of chlorophyll *a* may be represented as follows:

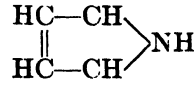
$$C_{32}H_{20}ON_4Mg\begin{cases}COO\cdot CH_3\\COO\cdot C_{20}H_{39}\end{cases}.$$

Phytol extracted in a pure form is a colorless liquid.

The structure of the central nucleus of chlorophyll is of great interest. Omitting details that would lead into physiological chemistry instead of physiology, it is necessary to mention that the atom of magnesium occupies in it a central position and is connected with the atoms of nitrogen according to the formula:



The atoms of nitrogen in their turn constitute a part of the heterocyclic structure of the pyrrole group, which is characterized by a five-membered ring of atoms:



This establishes a certain chemical relationship between chlorophyll and the blood pigment hemoglobin in whose structure the pyrrole groups also occur.

The structure of chlorophyll has been established through a detailed study of the successive disruption of its molecules under the influence of alkalies, acids, etc. The action of alkalies induces in the first place the saponification of the ester groups, *i.e.*, the splitting off of phytol and methyl alcohol.

The products of reaction are the alkali salts of chlorophyllinic acids, retaining the undisturbed structure of the central nucleus as well as the color and fluorescence of chlorophyll. The action of acids results in the splitting off of magnesium from the chlorophyll molecule and in the formation of a brown product without any fluorescence. This brown color is frequently observed when leaves containing a considerable amount of acid in their cell sap are killed, *e.g.*, leaves of sorrel or *Begonia*, from which it is almost impossible to obtain unmodified chlorophyll. Willstätter succeeded in showing that, when carefully treated with acid, the molecule of chlorophyll remains almost unchanged, only hydrogen replacing magnesium. This substituted chlorophyll he called "phacophytin." The green color may be restored if the hydrogen is replaced by magnesium, zinc, copper, or iron. When acted upon by stronger acids, the hydrolysis of the other groups and the further breaking down of the complex molecule of chlorophyll take place.

It is interesting and important to note that having investigated over 200 species of plants, Willstätter has found in all of them the same chlorophylls. Hence, this most important substance seems to be uniformly the same in all plants. From this, the conclusion may be drawn that the fundamental chemistry of the process of carbon assimilation is also similar in all chlorophyll-bearing organisms.

Besides chlorophylls, the green plastids of the leaf contain two other classes of pigments, carotenes and xanthophylls. These

are yellow in color. They may be extracted, together with chlorophylls, by treating green leaves with alcohol. Their presence in the alcohol extract is readily detected after shaking the solution with benzine. The chlorophylls, which are more soluble in benzine than in alcohol, accumulate in the upper benzine layer, while the xanthophylls remain in the alcohol, coloring it yellow (Kraus's reaction). A complete separation of the pigments, however, is not obtained, as the carotenes pass over into the benzine along with the chlorophyll and must be isolated in a much more complicated way.

Carotene exists in several forms, all of which are unsaturated hydrocarbons of the formula $C_{40}H_{56}$. These are readily oxidized on exposure to air. Xanthophyll contains 2 atoms of oxygen and has the formula $C_{40}H_{56}O_2$. In its composition, it is similar to carotenes, although it is not merely a product of their oxidation and not easily convertible into carotenes.

Carotenes and xanthophylls, as well as related carotenoid pigments, give a yellow color to the chromoplasts in many fruits and in flower petals. Lutein, the pigment of the yolk of the hen's egg, is closely related to xanthophyll and is isomeric with it in its empirical formula. The color of the tomato fruit is produced by lycopene, an isomer of carotene.

The physiological importance of the yellow pigments of the chloroplasts is not understood, but according to present views, they do not take a direct part in carbon assimilation. Some authors assume that the yellow pigments, which are always present in the chloroplasts, act as screens or light filters, thus protecting the chlorophyll against rapid destruction by light. Others believe that the yellow pigments are the carriers of the oxygen formed in assimilation, taking it from the peroxide-like combination of chlorophyll with carbon dioxide (see Art. 43), which forms during the early stages, and afterward giving it up to the atmosphere, thus promoting photosynthesis. These hypotheses, however, have not been sufficiently established. There is a possible relationship between carotene and sexual reproduction in plants.

Of great interest is the close connection of the chemical composition of carotene and vitamin A. The long hydrocarbon chain of carotene ruptures at the center linkage into two equal halves; with the addition of oxygen, each half forms a molecule

of vitamin A. Carotene can be regarded as provitamin A, and this explains the high dietetic value of vegetables, especially of carrots, which contain much carotene, as do also lettuce, spinach, and other leafy vegetables.

40. Conditions of Chlorophyll Formation. Etiolation and Chlorosis.—The formation of chlorophyll is dependent upon several definite requirements. Of these, one of the most important is the presence in the cells of plastids, in which chlorophyll, a green pigment, is synthesized. Chlorophyll is not formed in roots, except in the aerial roots of plants such as the tropical orchids. Another important requirement for the formation of chlorophyll is light exposure. Leaves and stems developed in darkness remain almost white or are colored yellow by carotenes and xanthophylls. Such plants are said to be etiolated. They turn green rapidly when exposed to light. It is supposed that they contain a special substance called "leucophyll," which is colorless but readily changes into chlorophyll under the influence of light. The nature of this "chromogen," as substances easily turning into pigments are called, has not been investigated. In some cases, *e.g.*, in the germinating seeds of conifers, chlorophyll may form also in the dark.

Besides their pale hue, etiolated plants differ from normal ones also in respect to their form. The aerial organs usually grow out very long, while the leaves, on the contrary, are undeveloped (Figs. 50 and 51). This modification in form has no direct connection with the absence of chlorophyll but is due to the growth of cells and tissues in the absence of light.

Another important requirement for chlorophyll formation is the presence of a minimal amount of iron salts in the cells that synthesize it. Plants grown in water cultures, in solutions carefully purified of all traces of iron, have a pale yellow color. Under these circumstances, the process of assimilation goes on feebly, or not at all, and the plants finally die from exhaustion. But if the leaves of such plants are sprayed with a solution of an iron salt, green spots are produced in places where the salt has penetrated. Localized green color may also be produced when part of the roots of a water culture are separately supplied with iron. When thus treated, parts of the plant drawing directly on these roots will turn green.

The necessity of iron for the formation of chlorophyll was for a long time regarded as a strong proof that iron is one of the constituents of chlorophyll. The exact analyses performed by Willstätter have shown that this view is not correct. The iron must be regarded, therefore, as a specific catalyst without which there cannot go on some of the preliminary stages of the produc-



FIG. 50.—Seedlings of broad beans grown in light (*from Nathansohn*).



FIG. 51.—Seedlings of broad beans grown in darkness (*after Nathansohn*).

tion of green pigment, like the formation of leucophyll or other precursors.

It is always possible by means of microchemical reactions to detect in the chloroplasts considerable amounts of iron, which, according to Warburg, plays a very important role in the process of photosynthesis. This theory will be discussed later on.

Chlorosis is sometimes observed under natural conditions, most frequently on soils rich in lime and giving a somewhat

alkaline reaction. Evidently, this may be explained by the fact that under these conditions, the iron salts are insoluble and cannot be absorbed by the roots. Some plants, *e.g.*, lupines and tobacco, are very much subject to chlorosis on such soils. Chlorosis may be induced also by other causes that check the normal supply and translocation of mineral salts in the plant. In some southern crop plants (tea, pomegranate), chlorosis is observed in the spring. It is induced by the low temperature of the soil, which inhibits the activity of roots.

Chlorosis is produced also in cases of infections due to diseases. Sometimes it is localized, when the leaves show green as well as colorless portions, as in mosaic diseases of tobacco, potato, etc.

The phenomenon of chlorosis must not be confused with albinism. The latter is observed when a plant, owing to hereditary causes, is not able to produce chlorophyll even under the most favorable external conditions. Such albino plants may show no chloroplasts on microscopic examination. Frequently among the seedlings of corn and of other cereals, pale yellow specimens are observed, which of course cannot assimilate carbon dioxide and therefore die from starvation after the development of two to three leaflets. Other plants may show partial albinism, when some of the leaves, or separate portions of the leaf, may be whitish. Such plants with variegated leaves are much valued in decorative horticulture. In the colorless portions of such leaves, an increased amount of oxidizing enzymes will usually be found. But whether this increase in oxidation is the direct cause of the inability to accumulate green pigment, or whether it is but an accompanying phenomenon, is not known.

41. Importance of the Energy of Light for Carbon Assimilation. The Role of Chlorophyll in the Absorption of Light. Optical Properties of Chlorophyll.—The mere presence of green plastids does not assure carbon assimilation. Light is an essential condition for this process. As early as 1779, Ingen-Housz definitely established that only in the presence of light do plants "improve the air." Light is the only source of energy that can be used to break the strong linkage between oxygen and carbon that is necessary for the production of carbohydrates from carbon dioxide. To be available in the process of photosynthesis, the energy of light in the first place must be absorbed by the chlorophyll and transformed into chemical energy. Chlorophyll

absorbs light selectively; part of the wave lengths are transmitted. If sunlight is passed through a chlorophyll solution and then spread by means of a prism into its constituent wave lengths, portions of the spectrum are absorbed by chlorophyll. Absence of the wave lengths absorbed will cause dark bands; while others that are transmitted appear as bright bands of the hues of the spectrum, some of which will be transmitted in different degrees. In this manner, the absorption spectrum of chlorophyll is obtained (Fig. 52).

The most intense absorption takes place in the region of the red and orange rays, between the Fraunhofer lines *B* and *C*

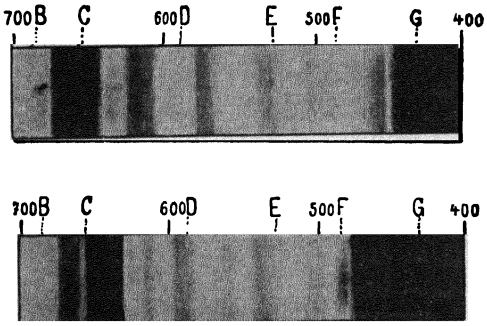


FIG. 52.—Absorption spectrum of chlorophyll. Above, chlorophyll *a*; below, chlorophyll *b* (*after Willstätter and Stoll*).

(wave length, 630 to 680 $\mu\mu$). An almost equally intensive absorption will be found in the blue-violet portion of the spectrum, to the right of line *F* (470 $\mu\mu$)($1\mu\mu = .001\mu$). In addition to these, several other less distinct absorption bands may be detected. The absorption spectra of chlorophyll *a* and *b* differ somewhat. In *b*, the absorption in the blue-violet region covers a somewhat larger region, while in the red-orange portion the area is smaller.

As the concentration of the chlorophyll increases, the absorption bands become broader and merge (Fig. 53). Finally, there are two absorption bands: one on the right side of the spectrum, covering the red and yellow rays; the other on the left part of the spectrum, embracing the blue-violet rays. The green and part of the red rays that lie at the lower limit of visibility can pass without being absorbed. It is the combination of these two spectral portions that imparts to the chlorophyll the green

color p[illegible] to it. With further increase in concentration, the green rays also are completely absorbed. Finally, only the dark-red rays remain visible. A very thick layer of a chlorophyll solution is no longer green but brick red.

Besides the selective absorption of light energy, chlorophyll possesses another important optical property, fluorescence. In reflected light, it appears blood red, owing to the fact that a part of the rays falling on it are transformed and reflected with an

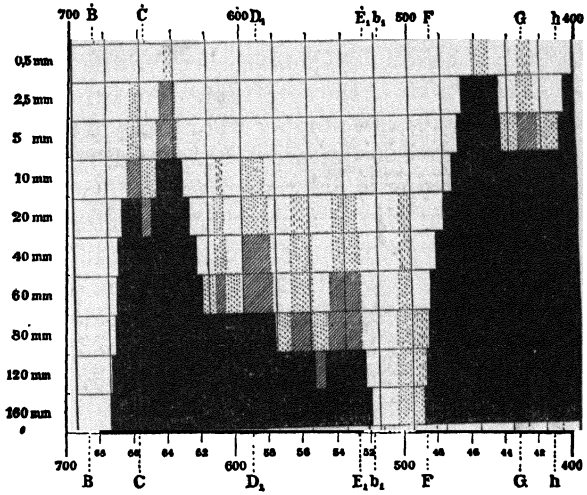


FIG. 53.—Merging of bands in the absorption spectrum of chlorophyll with increasing thickness of the solution layer (*after Willstätter and Stoll*).

altered wave length. The faculty of fluorescence points to a considerable photochemical activity in chlorophyll.

The complete mechanism is not clear by which chlorophyll renders the radiant energy that is absorbed by it effective in the process of decomposition of carbon dioxide. It has been assumed that chlorophyll plays the role of a sensitizer; *i.e.*, it has the property of rendering the energy of the absorbed rays effective for carbon dioxide decomposition, which cannot be accomplished by visible wave lengths but only by the ultraviolet. According to modern photochemistry, radiant energy shows some characteristics that allow it to be absorbed in definite units called "quanta." Every substance subjected to photochemical decomposition absorbs the radiant energy in a strictly defined quantity, 1 quantum or more per molecule. The quanta are constant

magnitudes for each kind of ray, but they vary in energy values with the length of the wave; the greater their wave length, the smaller the energy of each quantum. The energy of the quantum of light energy absorbed must be as large as, or larger than necessary to decompose the carbon dioxide. For this purpose, only the quanta represented by the ultraviolet are large enough to do this without chlorophyll. But chlorophyll has the ability to absorb 4 quanta at one time, so that the energy of activation of the chlorophyll can be made up of 4 small quanta, which then suffice for the decomposition of the carbon dioxide. Hence, the photocatalyst chlorophyll makes effective the small quanta found in the visible part of the spectrum. For light with a wave length of λ 486 (blue), this number will be 58.470 cal.; for λ 589 (yellow), 48.240; for λ 800 (infrared), 35.510 cal.

That the participation of chlorophyll in photosynthesis is not only of a physical but also of a chemical character is shown by the ease with which it decomposes when exposed to light. Alcohol and benzine solutions of chlorophyll when brought into contact with air are readily discolored by sunlight and even by diffused light. This may be due to the acceleration of the oxidation processes under the influence of light. Chlorophyll is much more stable in the living plastids of the plant, where photosynthesis is proceeding under strong illumination. The causes of this stability have not been discovered. Some authors are of the opinion that as the chlorophyll decomposes in the living plastid, it is re-formed. Some suppose that chlorophyll is not in a free state in the plastids but is combined with the protein substances of the stroma, which makes it more stable. Still others assume that chlorophyll is in a colloidal condition and therefore possesses different properties from those of a true solution. In favor of the last two theories is the observation that colloidal solutions of chlorophyll, obtained by highly diluting the alcohol solutions with water or by triturating the leaves with water, are much more stable when exposed to light than the usual solutions in alcohol or benzine.

42. Quantitative Relations between Absorbed and Stored Energy in the Plant. Decomposition of Carbon Dioxide by Energy Obtained from Oxidation. Chemosynthesis.—Of great importance is the question of how much the leaf profits from the radiant energy that it receives, what part of the latter is stored

as potential energy of the accumulated organic compounds, and what part is utilized in other processes or given off to the environment. Determinations of the radiant energy that falls on the leaf and that passes through it show that on the average about 75 per cent of it is absorbed, while 25 per cent, especially the green and red rays, are either reflected or transmitted. From this it does not follow, however, that all of the absorbed energy is utilized by the leaf in photosynthesis. The amount of energy that is actually used may be determined in various ways. The total quantity of matter accumulated by the leaf during a certain interval of time may be determined and compared with the amount of energy absorbed during the same time. The method of using the two halves of a leaf as checks, described previously in Art. 38, may be conveniently used for this purpose. The increase in dry weight per unit leaf area will show, as has been noted, the amount of dry matter accumulated. And if the heat of combustion is known or determined, it is possible to calculate the percentage of energy stored. A number of valuable studies have been made according to this method by Krasheninnikov. Another method is to evaluate the amount of carbon dioxide decomposed by the leaf during a certain period without injuring the leaf. Since the amount of energy required for this process is known, it is easy to compare it with the total amount of radiant energy absorbed by the leaf during a certain period. This procedure was used by the English scientists Brown and Escombe. The third method, employed by Purievitsch, is based on the fact that a leaf decomposing carbon dioxide absorbs a greater quantity of radiant energy than a similar leaf in an atmosphere free from carbon dioxide. The difference between these values will show the amount of energy utilized in photosynthesis. All these determinations give concordant results. The coefficient of utilization of radiant energy by the plant in photosynthesis has a comparatively low value. Usually from 1 to 5 per cent, and only in exceptional cases is as much as 10 per cent, of the total energy absorbed, used in photosynthesis. The remaining 90 to 99 per cent is transformed into heat and is utilized for the evaporation of water in transpiration.

Recent determinations made by Warburg with single-celled algae suspended in water have given a considerably higher coefficient of utilization of radiant energy absorbed by the chloro-

plasts. With feeble illumination, a coefficient of 50 or even 60 per cent was obtained. This important fact shows that the chloroplast is a very perfect photochemical mechanism. If under natural conditions the leaf of a plant photosynthesizes a much smaller percentage of the absorbed energy than its chloroplasts would be able to use, this must be ascribed to secondary causes that prevent the mechanism from working to full efficiency, such as the intensity being too great in direct sunlight for synthesis of the carbon dioxide available, or injurious effects on the plastid.

That the plant utilizes the photochemical capacity of the chlorophyll insufficiently is shown by another interesting fact. The amount of carbon dioxide decomposed per unit time and per unit weight of chlorophyll is variable. In plants with dark-green leaves, this amount, termed the "assimilation number," is relatively low, while in those with light-green leaves it is comparatively high. In one of his experiments with etiolated bean leaves that were exposed to light and had acquired gradually a dark-green color, Willstätter observed, on the first day, an assimilation energy per unit surface of 40 mg.; on the third day, 96 mg.; and on the fifth, 104 mg. Hence, while the green color was being produced, assimilation increased 2.5 times. But the amount of chlorophyll increased during the same period 16 times; while the assimilation number was falling continually from 133 on the first day, to 24 on the third, and to 13 on the fifth.

Similarly, as among different varieties of plants of the same species, distinguished from one another by darker or paler leaf color, it must not be expected that the varieties with darker leaves will assimilate more energetically than those with pale leaves, for in the pale-green leaves the assimilation number may be higher. Willstätter observed in one of his experiments that the rate of assimilation was the same in the dark as in the pale variety of elms, though in the former the chlorophyll content was ten times greater. A similar relation was obtained by Lubimenko with shade and light plants, the first of which had darker leaves. The considerable increase of the assimilation number, coincident with the decrease in amount of chlorophyll, shows that there is always a certain surplus of chlorophyll in the leaf.

The selective absorption of light by the chlorophyll indicates that the assimilation process is not equal in the different parts of the spectrum. It was established long ago that in the green rays, which are feebly absorbed by chlorophyll, assimilation is comparatively insignificant. Senebier noted, early in the nineteenth century, that when leaves were placed under double-walled bell jars and the space between the walls filled with colored liquids of different composition, in the yellow-red half of the spectrum, under a solution of potassium bichromate, the plants were assimilating more intensely than in the blue-violet half under a solution of copper sulphate in weak aqueous ammonium hydroxide solution.

Detailed studies on assimilation in different wave lengths were performed by Timiriazev, who proved that the maximum of assimilation lies in red-orange region (between the Fraunhofer lines *B* and *C*), which is most completely absorbed by chlorophyll. The blue-violet rays, though strongly absorbed, have a considerably smaller photosynthetic effect. Although violet wave lengths represent large energy quanta, they are not so efficient in the decomposition of carbon dioxide in the green leaf as the smaller quanta of red rays. For the same number of calories, the red wave lengths carry a greater number of quanta than blue rays; these induce the breaking down of a larger number of molecules and show a higher photosynthetic efficiency.

Photosynthesis is not the only means of creating organic substance. There are plants belonging to the group of bacteria whose source of energy for the synthesis of such compounds is not solar light but exothermic processes of oxidation of different substances, *e.g.*, ammonia, hydrogen sulphide, etc., which serve the bacteria as a source of energy. As such may be mentioned the nitrifying bacteria, which oxidize ammonia to nitrites and further to nitrates; sulphur bacteria, which oxidize hydrogen sulphide to sulphuric acid; hydrogen bacteria, which oxidize hydrogen to water; etc. The energy of oxidation is then utilized for the decomposition of carbon dioxide, for the building of organic substances, and for the formation of compounds of their protoplasm. Their synthetic activity, being based on the transformation of one form of chemical energy into another, is therefore termed "chemosynthesis." Of most importance in this group are the nitrifying bacteria, which live in soil and transform the ammonia formed in the process of the decay of

organic residues into nitrites and further into nitrates. The classical investigations of Vinogradsky (1889) have proved that this oxidation is accomplished by two groups of bacteria. The first group of nitrite bacteria includes the genus *Nitrosomonas*. These bacteria are small mobile cocci that oxidize ammonia to nitrous acid according to the equation:

$$2NH_3 + 3O_2 = 2HNO_2 + 2H_2O + 158 \text{ cal.}$$

The other group of nitrate bacteria includes the genus *Nitrobacter*, a nonmobile short bacillus that oxidizes the nitrous acid, prepared by the bacteria of the first group, into nitric acid according to the equation:

$$2HNO_2 + O_2 = 2HNO_3 + 38 \text{ cal.}$$

It is obvious from these equations that the second process gives much less energy than the first oxidation step. Nitrite bacteria oxidize on the average 35 molecules of ammonia for the assimilation of 1 atom of carbon, while the nitrate bacteria require for the assimilation of the same amount of carbon 135 molecules of nitrous acid.

By the oxidation of nitrogenous compounds as their source of energy, and using carbon dioxide of the air as their source of carbon, the nitrifying bacteria do not require any other organic compounds as respiratory materials, and they are not capable of using them in respiration or synthesis. Moreover, organic substances indispensable to the respiration of most organisms, including even the sugars, are toxic to nitrifying bacteria. In spite of this singularity in metabolism, these bacteria are composed of the same compounds as other plants; *e.g.*, they contain the same protein substances. That carbohydrates participate in the structure of their protoplasm is an hypothesis that remains as yet insufficiently investigated.

Nitrifying bacteria represent a special physiological type, sharply differing from the green autotrophic plants as well as from the usually colorless heterotrophic saprophytes and parasites. The exothermic process of oxidation of ammonia into nitrous acid is used by the nitrifying bacteria both for the primary synthesis of organic substances and for the maintenance of other vital processes, such as growth and movement; such processes are maintained in other organisms, autotrophic as well as hetero-

trophic, by respiration. Between photosynthesis, or the synthesis of organic substances using the energy of light, and respiration, *i.e.*, the catabolic processes that have been emphasized in relation to the higher plants, the contrast is not so distinct in the nitrifying bacteria. The only means of providing the energy necessary for their metabolism is the oxidation of substances that are not constituents of their protoplasm but such reduced inorganic substances as ammonia and nitrous acid.

The nitrifying bacteria are very important in the general cycle of nitrogen in nature. These bacteria are present in all soils, except those that are waterlogged. Almost all of the nitrates that exist in natural conditions are the product of their activity. A peculiarity of these organisms is the strictly coordinated work of both groups of the nitrite and the nitrate bacteria, which are so closely interdependent that it was only with difficulty that Vinogradsky succeeded in separating them.

Vinogradsky also discovered the physiology of another group of organisms that obtain their energy from the oxidation of inorganic substances, the sulphur bacteria. The oxidation of hydrogen sulphide by these organisms proceeds according to the following equation:

$$H_2S + 2O_2 = H_2SO_4 + 115 \text{ cal.}$$

Just as in the case of nitrifying bacteria, the energy released is used for the various vital processes, and particularly for the decomposition of carbon dioxide. These bacteria therefore do not require organic substances for their nutrition. However, they differ from the nitrifying bacteria in that they are not injured by organic compounds in the nutrient medium.

The sulphur bacteria represent a fairly diverse group of organisms. They contain single and filamentous forms. Some of them possess a purplish-red pigment, bacteriopurpurin, and have been given the name of "purple bacteria." Engleman suggested that this pigment substitutes for chlorophyll and participates in the photodecomposition of carbon dioxide. Light, which generally suppresses the development of bacteria, exerts a favorable influence upon the purple bacteria. The physiology of the purple bacteria is not quite clear as yet. According to recent investigations, the reduction of CO_2 is closely interrelated with the oxidation of H_2S according to the equation

$$CO_2 + 2H_2S = CH_2O + H_2O + 2S..$$

There is no liberation of oxygen during this process. Besides nitrifying and sulphur bacteria, there is a series of microorganisms, the iron bacteria, which obtain energy from the oxidation of ferrous iron. These bacteria also were originally described by Vinogradsky. They oxidize ferrous ion Fe^{++} to ferric ion Fe^{+++}. Most organisms of this group belong to filamentous types, such as the genera *Leptothrix*, *Crenothrix*, etc. Their role in nature is exceedingly important. Their activity explains the formation of bog iron ore, large accumulations of iron oxide that occur in marshes and lakes. Some of these bacteria, however, are capable of using organic substances and thus in contrast to nitrifying bacteria do not represent obligate autotrophic organisms. Many of them are capable of oxidizing not only ferrous but also manganous ions.

43. The Chemical Reactions of Photosynthesis. Theories of Baeyer, Willstätter, and Warburg.—Although the equation for photosynthesis can be written thus,

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2,$$

and although no intermediate compounds have been demonstrated, still this simple equation must undoubtedly proceed through several steps. This is confirmed by the great complexity of the carbohydrates formed, which certainly cannot arise directly from such simple substances as CO_2 and H_2O; and further, the picture of photosynthesis is obscured by the difficulty of the reduction of such a stable compound as carbon dioxide. It has already been seen what great difficulties the analysis of the separate respiratory reactions involves. Photosynthetic reactions are still more complicated, for, in addition, light energy participates in the dynamics of the chemical processes. It is not surprising that as yet there is very little known of the separate phases of the process of photosynthesis and that there are several theories that are not completely coordinated.

One of the facts that should form the basis of every theory of photosynthesis is the nearly complete equality always observed in the volumes of the gases, oxygen and carbon dioxide, interchanged in photosynthesis.

Carbohydrates must always be the products of photosynthesis, because the proportion O_2 to CO_2 can be equal to unity only

with the formation from CO_2 and H_2O of those substances in which hydrogen and oxygen are in the same proportion as in water.

The formaldehyde theory advanced by Baeyer in the seventies of the last century fully meets these requirements. According to this theory, the first product of assimilation of carbon dioxide and water is formaldehyde.

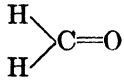
$$CO_2 + H_2O \rightarrow H{\cdot}CHO + O_2.$$

The formaldehyde obtained may then be polymerized under the influence of alkalies into a hexose as was first observed by Butlerow.

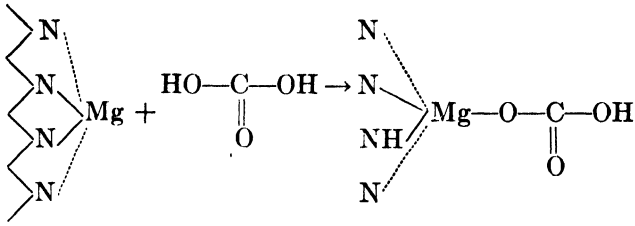
$$6H{\cdot}CHO \rightarrow C_6H_{12}O_6.$$

Against Baeyer's theory, arguments were advanced that formaldehyde is a very toxic compound; that it is used with great difficulty by plants for the synthesis of carbohydrates, and that therefore it can hardly be considered as an intermediate product of assimilation. These objections, however, could not prevent this theory from being generally accepted; for formaldehyde as an intermediate product does not accumulate in concentrations that could be toxic, while its nonavailability for synthesis when introduced into the cell artificially may depend upon its presence in too great concentration.

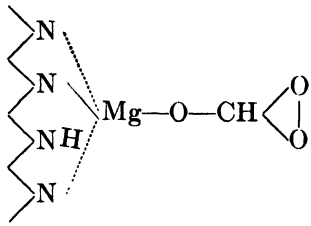
But Baeyer's theory has another weak point. It does not explain the most difficult transformation occurring during photosynthesis, *viz.*, the change of carbon dioxide, O=C=O, into the highly reduced compound, formaldehyde.



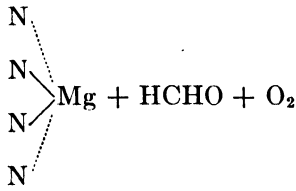
It is in this reduction that light energy is effective. Since the photochemical reduction of carbon dioxide requires the presence of a compound that can absorb light energy, such as chlorophyll, and taking into consideration recent chemical data, Willstätter revised the scheme given by Baeyer. According to this idea, first a hydrate of carbon dioxide is combined with magnesium of the nucleus of chlorophyll according to the following procedure:



This compound of chlorophyll and carbon dioxide directly absorbs light energy and is further transformed into the peroxide form.



Finally, the peroxide decomposes under the influence of enzymes, present in the stroma of the chloroplast or in the protoplasm, releasing free oxygen and formaldehyde, while chlorophyll is reduced to its initial form.



Willstätter's theory, explains the sensitizing action of chlorophyll. It is supported by the fact that the dry substance of the green leaf absorbs carbon dioxide almost as avidly as venous blood or alkaline solutions. The decomposition of the peroxide compound is accomplished by an enzyme belonging to the group of catalases, *i.e.*, enzymes that split hydrogen peroxide with the liberation of molecular oxygen.

Photosynthesis, therefore, may proceed through three phases at least: the union of carbon dioxide to chlorophyll, the internal rearrangement of atoms in the compound with transformation into a peroxide form, and the decomposition of the peroxide

with liberation of free oxygen. Of these three phases, only the middle one represents a photochemical process taking place with the absorption of light energy. The other two phases are purely chemical processes. Such a complexity of the photosynthetic process explains the fact long ago established that photosynthesis follows van't Hoff's temperature rule, that with every 10°C. increase in temperature the rate of the reaction doubles. Now a characteristic feature of purely photochemical reactions is their almost entire independence of temperature. The effect of temperature on photosynthesis will be comprehensible if it is assumed that it is built up of three phases, of which two represent "dark" reactions, *i.e.*, chemical reactions that proceed without the participation of light.

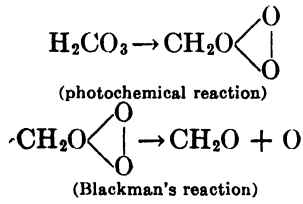
Warburg's concept of photosynthesis approaches in some degree the scheme of Willstätter. It is based chiefly on his study of photosynthesis of the unicellular alga *Chlorella*. The simplicity of the structure of this alga makes it possible to detect more easily many of the important relations during the process of photosynthesis.

According to Warburg, photosynthesis should be regarded first of all as a reaction proceeding on the surface of the chloroplasts. This is indicated by the fact that the process is strongly hindered by the influence of surface-tension reducing agents. According to this view, chlorophyll is distributed on the surface of the chloroplasts in a very thin layer 1 molecule in thickness, a monomolecular film, and it is in this layer that the carbon dioxide is decomposed. The decomposition proceeds in two phases, of which the first represents a photochemical reaction. It is independent of temperature and proceeds with extreme rapidity, according to Emerson's measurement in 0.00001 sec. The second phase is designated by Warburg "Blackman's reaction," (named in honor of the English plant physiologist F. F. Blackman, who has contributed much to the understanding of photosynthesis). This is of a purely chemical nature and proceeds without the participation of light. It requires at 25°C. about 0.04 sec.; this chemical reaction is considerably slower than the physical reaction of the first phase.

Blackman's reaction is impeded by hydrocyanic acid and by hydrogen sulphide. According to Warburg, this indicates that here is involved the catalytic influence of iron ions, which

display high sensitivity to the reagents mentioned. In its character, Blackman's reaction approaches the catalytic decomposition of peroxides, especially of hydrogen peroxide, occurring under the influence of catalase and in which likewise ions of iron play a role.

Warburg represents his theory by the following equations:



As according to Willstätter's theory, the last phase of photosynthesis also consists in the splitting off of oxygen from the peroxide compound, both Willstätter's and Warburg's theories may be united into one general scheme, the last phase of Willstätter's scheme representing Blackman's reaction.

Both theories, Willstätter's as well as Warburg's, are to a considerable degree hypothetical and as yet are not quite complete. Their advantage when compared with Baeyer's and other purely chemical schemes lies in the fact that they explain the necessity of the whole complex for photosynthesis: chlorophyll + living stroma + protoplasm with all its enzymes and specific structure, and the impossibility of reproducing photosynthesis in solutions of chlorophyll.

That is the reason why repeated attempts to explain the course of photosynthesis by means of a study of chemical reactions occurring in water solutions of carbon dioxide under the influence of ultraviolet rays, electric discharges, and the like, must be recognized as grossly mechanistic and having no direct relation to what proceeds during photosynthesis in the green plastids of the plant.

44. Structure of the Leaf as an Organ of Photosynthesis. Penetration of Carbon Dioxide into the Leaf.—The process of photosynthesis takes place in the chloroplasts. Hence, in order that the carbon dioxide may serve as material for the synthesis of carbohydrates, it must be absorbed by the chlorophyll-bearing cells. These cells, forming the mesophyll of the leaf, are not in direct contact with the outer atmosphere, as, on either side, the

leaf is covered by an epidermis containing no chloroplasts and along its outer wall by a cuticle that does not permit mass movement of gases (Fig. 54). Carbon dioxide penetrates into the leaf and reaches the absorbing cells mainly through open stomata. The cuticle allows carbon dioxide to pass through it, but only in solution in the cuticular substances. The stomata are numerous small openings in the epidermis, whose structure is known from anatomical studies of plants. That the carbon dioxide enters the leaf mainly through the stomata may be shown by a simple experiment. If on a certain portion of the leaf the stomata are

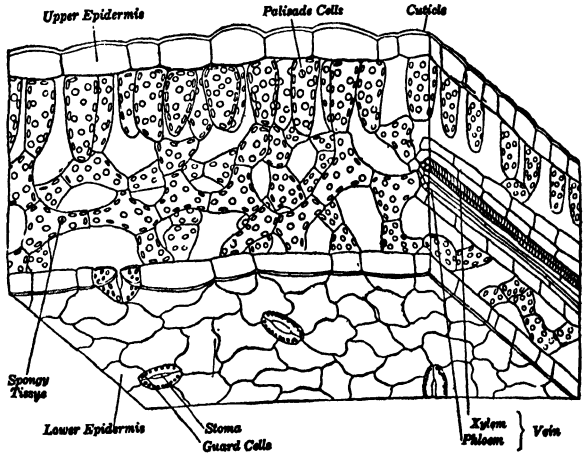


FIG. 54.—Cross section of a sunflower leaf (*after Smith et al.*).

coated with vaseline, and the leaf is then exposed to light and afterward treated with iodine, the blue color reaction will be observed only in those portions where the stomata remained open (Fig. 55).

It is well known that the degree of opening of the stomata is not always the same. In many plants, they are able to widen and narrow their apertures or close them entirely. These changes in the degree of opening have an influence on photosynthesis. Therefore, environmental conditions that induce the closing of the stomata, such as lack of moisture, also check photosynthesis. During periods of drouth, plants may give indications of starvation. They cease to develop and even lose in dry weight. This explains the poor development of plants in dry regions, as well as the low yields in years of drouth.

Assimilation is likewise suspended in conifers during the cold season except during short periods of warm weather or when the leaves are warmed in sunlight. Though they preserve their leaves, these organs are inactive during most of the winter, when the stomata are closed.

Even in the fully opened condition, the stomata occupy but a small part of the leaf surface, not more than 1 to 5 per cent of the whole area of the leaf. It might be thought that the diffusion of carbon dioxide into the leaf must be greatly impeded, as the gas has to pass through very small openings. Brown and Escombe showed, however, that an epidermis, perforated by numerous small openings, is less of an obstacle to the diffusion of carbon dioxide than might be supposed. In an experiment conducted with the leaf of *Catalpa*, it was observed that 1 sq. cm. of the leaf's surface absorbed 0.07 cc. of carbon dioxide per hour. An equal surface of an alkaline solution absorbs during the same period 0.12 to 0.15 cc. of CO_2, or only twice as much. It must be noted, however, that of 1 sq. cm. of leaf surface only 1 sq. mm., or one-hundredth of its area, is occupied by the stomatal openings, the remaining 99 per cent being cuticle. It may be calculated from this that the carbon dioxide penetrates into the stomata at a rate fifty times higher than the rate of absorption by a solution of alkali having an area equal to the area of the stomatal openings.

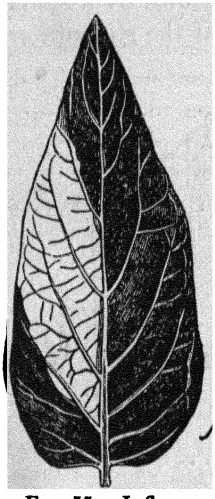


FIG. 55.—Influence of coating of stomata on the formation of starch in the leaf (*from Palladin*).

This seeming paradox finds its explanation in the peculiarity of the diffusion of gases through small openings. According to Stefan's law, the rate of diffusion through orifices is proportional to their area only when their dimensions are very large. With very small openings, the rate of diffusion is a function of their diameters.

As the number of stomata in a leaf is very large, usually several hundred per square millimeter, the sum of their diameters, in spite of their small size, is considerably greater than the diameter of the leaf. Therefore, diffusion through the membrane perforated with stomata proceeds almost at the same rate as if this

membrane did not exist and the absorbing cells of the leaf were in direct contact with the atmosphere.

Willstätter showed by experiment that in a saturated atmosphere when the cells of the leaf mesophyll are protected from drying, photosynthesis proceeds with the same velocity both in uninjured leaves and in leaves with the lower epidermis removed. Thus the epidermis with open stomata does not check in the least the penetration of carbon dioxide into the leaf. Gas diffusion through the stomata, as well as the movements of the stomatal apparatus that regulates the communication of the intercellular spaces with the surrounding atmosphere, will be discussed in the chapter devoted to the water balance of plants.

In order to give an idea of the number and dimensions of stomata, the values obtained by Kiesselbach as a result of the investigation of 11 varieties of corn are presented in the following table.

Number of stomata per square centimeter of lower epidermis	7,684
Number of stomata per square centimeter of upper epidermis	9,300
Total for both surfaces	16,984
Total number of stomata per plant (approximate)	104,057,850
Total leaf surface per plant (approximate)	6,100 sq. cm.
Average dimensions of one stoma, microns	25.6 × 3.5
Average area of stomatal openings, square microns	89
Area occupied by stomatal openings (in percentage of total area of the leaf)	0.76 per cent

Having passed through the stomata, the carbon dioxide enters the intercellular spaces of the leaf and diffuses through the walls of the chlorophyll-bearing cells. In the majority of plants, on the upper side of the leaf is situated the palisade tissue, consisting of closely fitting cells that are arranged perpendicular to the leaf surface. As these cells are rich in chloroplasts, the palisade tissue must be regarded as being the primary assimilating tissue. Closer to the lower epidermis lies the spongy parenchyma with loosely arranged cells, forming large intercellular spaces whose dimensions frequently surpass those of the cells. These cells contain fewer chloroplasts and are less active in assimilation. The spongy tissue is regarded as being especially fit for

the aeration of the leaf. The stomata are usually arranged on both sides of the leaf in herbaceous plants that are adapted to exposed habitats. In trees and in shade plants, the stomata are situated most often on the lower side, the upper epidermis being without them.

Having reached the surface of the chlorophyll-bearing cells, carbon dioxide dissolves in the water with which the cell walls are impregnated and then diffuses in the form of H_2CO_3 or, more correctly speaking, in the form of bicarbonate, HCO_3^- into the protoplasm toward the green plastids, which absorb it.

The absorption and assimilation of carbon dioxide are favored by the fact that the chlorophyll, which according to Willstätter's scheme combines chemically with carbon dioxide, is situated on the surface of the chloroplast.

The total surface area of the chloroplasts is very great indeed. According to Schröder's calculations (1926), the total surface area of the chloroplasts of a beech tree 100 years old is about 200 times the area of the leaves. Such an enormous surface for absorption is of great significance for photosynthesis; for the plants are compelled to obtain the carbon dioxide required from the atmosphere, which contains it in quantities less than 0.5 mg. per liter, or 3 parts in 10,000 of air. To secure the normal progress of assimilation, the cell walls must be sufficiently saturated with water. Since the sun's rays induce a continuous evaporation of water from the leaf, to supplement these losses the leaf possesses a dense network of bundles that carry water. They communicate through the petiole directly with the xylem tissues of the stem and the roots. The phloem of these bundles serves for the translocation of the products of assimilation, which is likewise of major importance for uninterrupted photosynthesis. Experiments show that leaves separated from the plant and thus deprived of the possibility of translocating the products of assimilation, with continuous illumination are finally so filled with them that further photosynthesis is impeded.

The translocation of assimilates from their place of origin, the chlorophyll-bearing cells of the leaf, is favored by the shape of the cells. They are elongated perpendicularly to the surface of the leaf in the palisade tissue. At their lower ends, the palisade cells join special collecting cells, which in their turn communicate with those of the phloem. Thus a continuous

flow of the products of assimilation is established from the parenchyma cells to the veins of the leaf and further from the veins through the petiole to the stems and other organs of the plant.

45. Dependence of Photosynthesis on Light Intensity. Sun and Shade Plants. Culture of Plants in Artificial Light.—Light being the source of energy for photosynthesis, it is natural that with increased intensity of light the process also goes on at a higher rate, but this does not hold true for high light intensity. If, beginning with low values, the intensity of light is augmented by steps representing equal energy increases, assimilation will

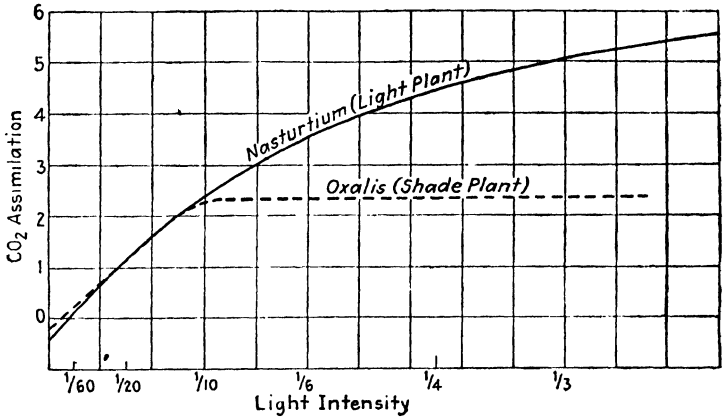


FIG. 56.—Dependence of assimilation on the intensity of light in a light plant (Nasturtium) and a shade plant (Oxalis) (*after Lundegårdh*).

increase by about uniform steps until a certain intensity of light is reached. Further increase of light intensity then induces no increase of photosynthesis. If the dependence of carbon assimilation on the intensity of light is represented graphically, marking on one axis the quantity of carbon dioxide that is decomposed during a time unit, and on the other the intensity of light in fractions of direct sunlight falling on the surface of the earth during the noon hours of a bright summer day, it will be seen that the curve of the dependence of assimilation on the intensity of light will show in general, a logarithmic character (Fig. 56).

Not in all plants, however, is the dependence of assimilation on the amount of light expressed by a curve of this nature. In those peculiar to open, sunny habitats, assimilation increases

until the intensity of light has attained the intensity of full sunlight (the continuous curve in Fig. 56). In plants adapted to shaded habitats, *e.g.*, *Oxalis* (the broken curve in Fig. 56),

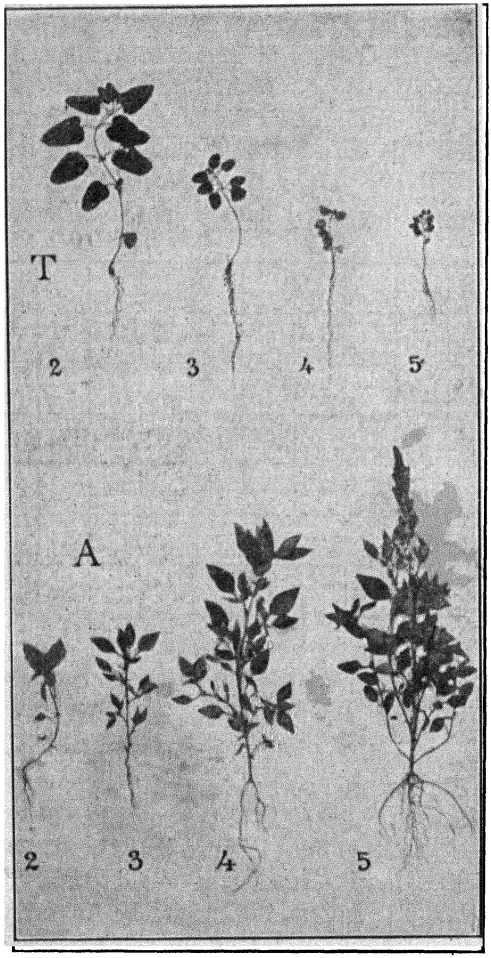


FIG. 57.—Development of the shade plant *Teucrium scorodonia* (above) and of the heliophilous plant *Amaranthus retroflexus* (below) under different light conditions. The figures under the drawings show the growth under intensities increased in the ratios 2:3:4:5, the last being full sunlight (*redrawn from Combes*).

assimilation increases until light has attained a comparatively low intensity, or about one-tenth of full illumination. Further increase of light remains without effect or even induces a depres-

sion of photosynthesis. In respect to their response to light intensity, all plants may be divided into two large groups, light plants and shade plants, but no sharp line of demarcation can be drawn between these two. They are connected by transition forms, or shade-tolerant plants.

Light plants develop the better the more they receive direct sunlight. They will not tolerate much shading, and they will grow, under natural conditions, exclusively in open places. The common weed of prairie regions, *Amaranthus retroflexus*, may be mentioned as an example of such plants. If this plant is deprived of but one-quarter of the total daylight, it is considerably checked

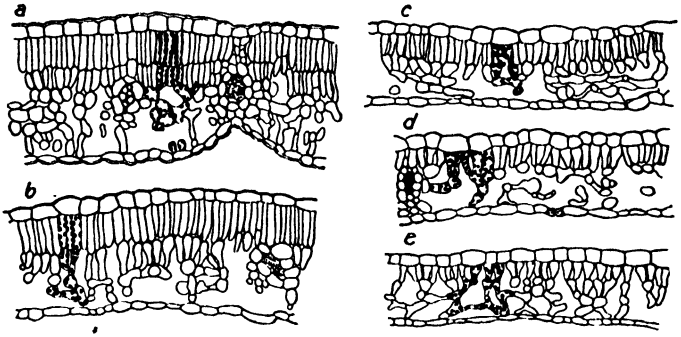


FIG. 58.—Leaves of the hazelnut: *a* and *b*, leaves exposed to the sun; *c*, *d*, *e*, shaded leaves (*after Hesselman from Lundegårdh*).

in its development and loses its capacity to bloom. On the other hand, shade plants, *e.g.*, *Teucrium scorodonia*, commonly found in the woods of western Europe, shows marked signs of depression when grown in open places. According to the experiments of Combes, *Teucrium* thrives when it receives but one-third of the total daylight, or under conditions where *Amaranthus* is hardly able to exist (Fig. 57).

Light plants differ from shade plants in many anatomical as well as physiological characteristics. The leaves of light plants are thicker. This thickness is produced by the greater development of the palisade parenchyma often arranged in two to three layers. Frequently, in such plants, the palisade parenchyma is formed not only at the upper side of the leaf but also at its lower side. Moreover, the cells are of smaller size, the number of stomata per unit surface is considerably greater, carbon dioxide

penetrates into the leaf more rapidly, and the network of conductive bundles is considerably denser. It is of interest to note that plants of the same species, when developing under different light conditions, acquire a different structure. Even leaves of the same tree will show marked shade or light characteristics (Fig. 58), depending on whether they are from the north or the south exposure, from the periphery or from the interior of the crown.

Shade plants differ from light plants also in regard to the amount of chlorophyll, showing a considerably higher content in the latter. Owing to this fact, they are able to utilize the small quantities of light that have filtered through sun-loving plants. Moreover, with the same light intensity, assimilation goes on in them at a higher rate than in light plants, as may

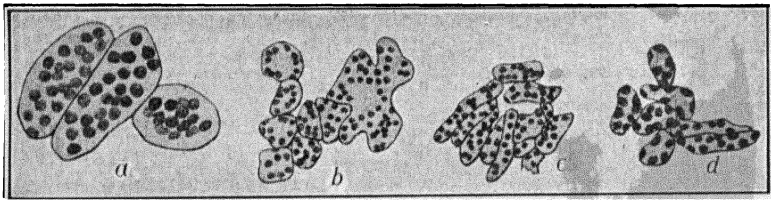


FIG. 59.—Comparative size of plastids in sun and in shade plants: *a*, Taxus (shade); *b*, larch (sun); *c*, locust (sun); *d*, beech (shade) (*after Lubimenko*).

be seen from the broken line in Fig. 56. But with high intensity of light, their thin leaves, which are insufficiently supplied with water by the fine network of vascular bundles, cease to increase their assimilative activity at an earlier moment, and the curve proceeds parallel to the horizontal axis of the coordinates. The chloroplasts of shade plants usually differ from those of light plants in their larger dimensions (Fig. 59).

It has been impossible as yet to find an exact numerical expression for the degree of light adaptation of a given plant, as in natural habitats they develop under light conditions that vary continuously in accord with the diurnal and annual fluctuation of solar radiation. One of the first attempts to ascertain the relative minimal amount of light in which plants are able to develop was made by Wiesner. He determined the intensity of light during the noon hours in the most shaded habitats, where certain plants occur, and compared it with the intensity of light in an open place. The fraction obtained shows with what part

oats, with a light intensity of 300 candle-meters; while the compensation point of the very shade-tolerant liverwort *Marchantia*, growing well in the deepest shade, lies at a light intensity of 100 candle-meters. Correspondingly, the respiratory intensity of mustard per 50 sq. cm. equaled 0.87 mg. CO_2 per hour; of oats, 0.37 mg.; and of *Marchantia*, 0.06 mg. The assimilation intensity of these three plants varies but little. The exact numerical expression of the amount of radiant energy required by the plant for its normal development can be obtained only when it is possible to grow normal plants entirely in artificial light under controlled conditions. Several such attempts have been made. By using the light of strong electric lamps of several thousand candle-power, it is possible to grow plants and to make them bloom and mature fruits (Fig. 60) in a room entirely deprived of daylight, for instance, in a cellar.

Some data may be given regarding the strength of illumination in candle-meters sufficient to induce flowering and fruiting of plants: peas, 1,100; beans, 2,400; barley and wheat, 1,800 to 2,200; radish, 4,000; tobacco, 2,200 to 2,800; corn, 1,400 to 8,000; buckwheat, 850 to 1,100; etc. (Harvey, 1921). By way of comparison, it should be mentioned that direct sunlight at noon gives about 30,000 to 40,000 candle-meters (9,250 foot-candles at 42 deg. N. Lat. on June 21 at noon). It is interesting to note that plants grown under conditions of artificial lighting develop much better when continuously illuminated during day and night than when subjected to an 8- or 12-hr. period of darkness. Hence, the "night's rest" is not necessary for plants. The possibility of work without rest for several months seems an amazing fact. But it must be kept in mind that in photosynthesis there is a continual input of energy and that rest as in animals is necessary only for repairs and for relaxation of the nervous system.

In the growing of plants in artificial light, the greatest difficulty lies in providing them with light, not only of a sufficient intensity, but also of an adequate quality, that is, of such wave lengths as permit the normal course of the photosynthetic process. The light of the strong incandescent electric lamps usually applied for illumination, as compared with daylight, is poor in rays of the blue-violet half of the spectrum, from 4000 to 5800 Å., and too rich in those of the yellow-red half, especially in infrared rays,

from 5800 to 7600 Å. and higher; that is why electric light appears somewhat yellow to the eye.

As photosynthesis is most intense in the orange-red region of the spectrum, the composition of artificial light is sufficiently favorable for the assimilation of carbon dioxide by the plants. But as it is poor in blue-violet rays, the processes of the formation



FIG. 60.—First cabbage and clover plants ever grown entirely in artificial light; used for carbohydrate analysis (*after Harvey*).

of organs deviate from their normal course in that the stems elongate excessively, the leaves on the contrary remaining insufficiently developed. Elongation is also favored by the abundance of infrared rays, which cause increased heating of the plants.

It must be mentioned that great divergencies exist among various kinds and groups of plants in their relation to the composition of light. Many crop plants, *e.g.*, wheat and other cereals,

flax, cucumbers, tomatoes, strawberries, beans, buckwheat, and others, thrive excellently in artificial light; while others such as radishes, cabbage, salad, spinach, and especially sunflower are extremely sensitive to the deficiency of blue-violet rays and elongate excessively under such conditions.

For the normal growth of plants, it is of major importance to improve the composition of artificial light by the addition of blue-violet rays. However, sufficiently powerful and convenient sources of these rays are not yet available. Arc street lamps are suitable as to the quality of their light if the high violet region is absorbed by a glass mantle, but they are not convenient to use. This led to the substitution of incandescent electric lamps. Mercury arc lamps also could serve as a source of the rays required. They contain mercury vapor, which with the passage of electric current emits light rich in green, blue, and considerable ultraviolet rays. Mercury arc lamps with a quartz tube allow the free passage of ultraviolet rays, but for plants these rays are injurious, and even a short exposure causes serious injuries to the leaves. That is why in adding to the incandescent lamps the light from mercury arc lamps it is necessary to provide them with a glass tube that absorbs the harmful shorter ultraviolet rays. Experiments carried out with such lamps have given favorable results with only a few plants. This may have been caused by a too low light intensity.

During recent years, tests have been made with the cultivation of plants in the light of neon lamps, which in the shape of tubes are used for signboards. When an electric current of high potential is passed through neon, it emits a reddish-orange light well utilized by plants in photosynthesis. Neon lamps have the advantage that about 80 per cent of the wattage consumed by them is transformed into light energy, while the efficiency of incandescent lamps is not more than 15 per cent. But being almost completely devoid of blue-violet rays, neon light does not foster normal development in plants and should be added only as a source of additional light to the normal daylight or to the light of incandescent lamps.

The growing of plants entirely in artificial light is undoubtedly very expensive. Most of the plants require on the average about 500 watts per square meter of the soil surface. With 24 hr. of illumination, this makes up 12 kw.-hr. per day. That is

why this method is applicable on a wide scale only in places where electric energy is very cheap, for instance, close to large hydroelectric stations, and only to the more expensive crops, *e.g.*, in the growing of vegetables rich in vitamins in polar regions or for scientific purposes.

Considerably less expensive and more profitable is the addition of artificial light to normal daylight during the winter months, when the day is too short and the light intensity too weak. In this case, smaller intensities of electric light applied during shorter periods suffice. Besides, several hours of natural daylight contribute to a more adequate formation of the plants. It is in the form of additional illumination that electric light is being widely applied in greenhouse culture and for scientific purposes. With such an addition, flowers and vegetables may be cultivated during the darkest winter months, and two to three and even four generations of crop plants instead of one may be obtained in the course of one year. This is of major importance for accelerating the production and selection of valuable hybrids.

46. Dependence of Photosynthesis on the Amount of Carbon Dioxide. The Carbon Dioxide Cycle in Nature.—The gradual lag in increase of photosynthesis in proportion to increased intensity of light depends not only on the fact that the chloroplasts are not able to use the excess of light energy, but also on the fact that the carbon dioxide absorbed by them per unit time is not sufficient for the full utilization of this energy. An increase of the carbon dioxide content in the atmosphere, therefore, always leads to greater photosynthesis. This may be seen from Fig. 61, which shows the increase in assimilation of pine needles, with the increase of the carbon dioxide content from 0.03 per cent, the normal amount in the air, to 0.28 per cent. It must be noted that the curve expressing the dependence of photosynthesis on the carbon dioxide content is of a logarithmic type, as was also the curve showing the dependence on light, and that this is the usual form of curves expressing the relationship of different physiological processes to the intensity of external factors.

Numerous investigations that have been conducted by different authorities seem to agree that the "normal" or "natural" content of the carbon dioxide of the air, *viz.*, about 0.03 per cent, is by no means the optimum but rather the minimum amount for the plant and that an increase of carbon dioxide often appears favor-

able.) On the basis of the above statement, fertilization with carbon dioxide has been applied during the past few years with a view toward obtaining higher yields. This was done first in greenhouses and afterward under the conditions of field experimentation, where carbon dioxide was conducted to the field by pipes. This method, of course, is practical only in the neighborhood of large factories, which throw into the air immense quantities of carbon dioxide. The Krupp factories in Essen, for instance, produce as much as 3,000,000 kg. of carbon dioxide per day. Especially favorable results have been obtained in

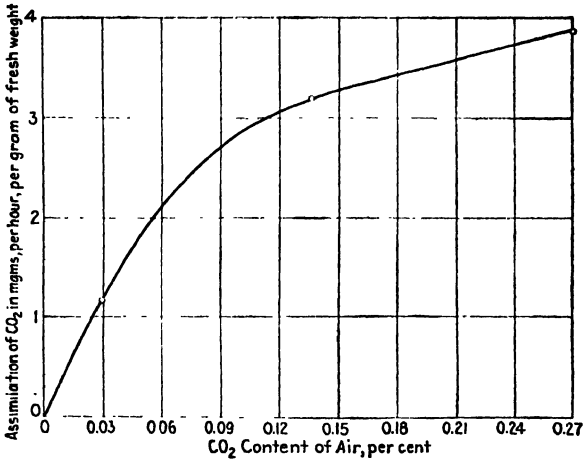


FIG. 61.—Dependence of assimilation of pine needles on the carbon dioxide content of the air (*after Stålfelt, from Lundegårdh*).

greenhouses, where, with insufficient aeration, often not even traces of carbon dioxide can be found in the air during the day. When this gas is introduced artificially, often a 100 to 150 per cent increase in yield has been secured. The rapid development of the plants in hotbeds underlaid with manure seems to be due not only to the higher temperature thus produced but also to the abundant supply of carbon dioxide.

Under natural conditions, plants obtain carbon dioxide not only from the atmosphere but also from the soil. Owing to the processes of decomposition of the organic substances in the soil by different microorganisms, carbon dioxide is liberated. Diffusing from the soil in the lower layers of the atmosphere, it is caught by the leaves of the plants. According to the calculations of

Lundegårdh, a sandy soil, poor in humus, liberates about 2 kg. of CO_2 per hour per hectare; loam and clay soils, containing a greater amount of humus, eliminate about 4 kg.; and forest soils, extremely rich in humus, produce from 10 to 25 kg. Moderately fertilized soils produce on an average 5 kg. of carbon dioxide per hectare per hour.

When the consumption of carbon dioxide by the plant is considered with the above figures in mind, the following values are obtained. A field of 1 hectare sown to oats consumes in the process of photosynthesis about 15 kg. of carbon dioxide per hour. Five of these are supplied by the soil; the other 10 are obtained from the atmosphere. In spite of the low percentage of carbon dioxide contained in the atmosphere, an air layer 100 m. thick and 1 hectare in area contains about 550 kg. of carbon dioxide. If it is assumed that in summer the most intense assimilation continues for about 8 hr., then the oat plants will consume in the course of a day 120 kg. of carbon dioxide per hectare. Of these, 40 are supplied by the soil and 80 absorbed from the air. This constitutes but 15 per cent of the total amount contained in a layer 100 m. thick. Lundegårdh observed that during the day the carbon dioxide content of the air is considerably lowered. At night, the "respiration" of the soil once more restores the balance, producing during 16 hr. the 80 kg. of carbon dioxide lost from the air.

This balancing of carbon dioxide takes place only in soils of medium fertility. In poor ground, plants absorb more carbon dioxide than is lost by the soil; hence, with the growth of the plants, the soil becomes enriched in humus. Conversely, in soils very rich in humus, the loss of carbon dioxide may exceed the accumulation of organic compounds by the plants. A specially great abundance of carbon dioxide has been found in forests under the cover of trees, where, in the lower layers of air, carbon dioxide may reach a concentration of 0.08 per cent instead of the average 0.03 per cent. This high percentage of carbon dioxide compensates the shade plants to a certain degree for the lack of light.

47. Dependence of Photosynthesis on Temperature. The Interaction of Several Factors and the Law of Limiting Factors. Diurnal Changes of Photosynthesis.—As has already been seen in discussing respiration and growth of plants, temperature

exerts a profound influence on all vital processes. Like chemical reactions, these processes follow van't Hoff's rule, according to which the rate of chemical reactions approximately doubles with every increase of 10°C. in temperature. One of the most characteristic features of vital processes is that due to the extreme complexity and instability of the protoplasm where these processes take place, they are subject to the rule of van't Hoff only within comparatively narrow limits of temperature, generally between 6 and 30 to 35°C. With further increase of temperature, the acceleration of the process is markedly decreased,

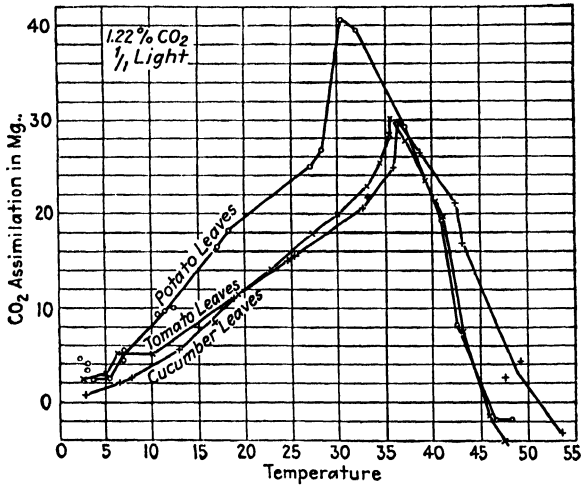


FIG. 62.—Dependence of assimilation on temperature, in the leaves of potato, tomato, and cucumber (*after Lundegårdh*).

after which a rapid falling off takes place, the bend in the coefficient curve often showing an acute angle (Fig. 62). At 40 to 50°C., the process completely ceases. Therefore, on the temperature coefficient curve of assimilation and other vital processes, three principal or, as Sachs called them, cardinal points are found; the *minimum*, at which the process just begins; the *optimum*, at which it goes on at its highest rate; and the *maximum*, after which the process stops again.

According to the ingenious explanation by Blackman, the sharp bend in the temperature curve shows that there are here not one but at least two processes to deal with. Both of them

are accelerated with increase of temperature, more or less in accordance with the rule of van't Hoff but inducing opposite effects in the leaf. If one of them is the process of decomposition of carbon dioxide and the other the process of the "inactivation," or loss of faculty, of the action of the chloroplasts, then with comparatively low temperatures the process of inactivation is almost imperceptible, and assimilation is subject to van't Hoff's law. But with a temperature of 20 to 25°C., the inactivation begins to increase in effect. It proceeds at a rapidly increasing rate, and at 30 to 35° it has overbalanced the process of assimilation and soon stops it. These relationships are shown diagrammatically in Fig. 63, where the curve *AB* shows the theoretical progress of assimilation as controlled by temperature; the curve *CD* shows the increase of the inactivation phase; and the curve *AE* indicates the actual progress of assimilation, as the resultant of the two curves.

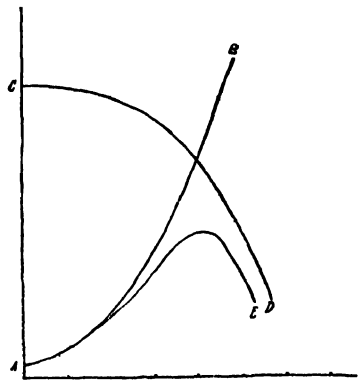


FIG. 63. Blackman's scheme, explaining the optimum in the temperature curve.

Blackman's explanation was completely confirmed by the experiments of his coworker, Miss Matthaei. In studying the character of the assimilation curve near the optimal point, Miss Matthaei discovered that the situation at this point depends on the duration of the experiment. The longer the experiment is continued, the lower will be the temperature at which the break in the curve will occur. From 37.5°C. in an experiment lasting for 1 hr., this point is moved to 30.5°C. in one that continues for 4 hr. This is quite plausible, as during a longer period the inactivation process becomes more effective.

In the light of this explanation, the conception of the optimum does not convey the implied meaning. Literally, this term means "the best temperature," while in reality at this point the factor of inactivation, or the destruction of the chloroplasts, is already considerable. A somewhat lower temperature should be recognized here as the "best" at which the above time factor of inactivation is as yet almost imperceptible.

The fact established by Blackman and his coworkers that photosynthesis follows van't Hoff's rule, though only within comparatively narrow limits, contradicted the view prevailing at that time that photosynthesis represents a purely photochemical reaction, for as such it is almost independent of temperature. As has been seen, this contradiction led Warburg and other investigators to the analysis of the process of photosynthesis into at least two phases, one of which is photochemical and does not depend upon temperature and the other purely chemical (Blackman's reaction) and thus subject to van't Hoff's rule.

The influence of temperature would show itself most clearly only when the experiments were conducted with sufficiently intense light and with sufficiently high concentrations of carbon dioxide. Under natural conditions, these two factors, especially a sufficient amount of carbon dioxide, are seldom secured. This circumstance complicates the experiment, and the primary influence of temperature on photosynthesis may be entirely disguised. In studying the simultaneous effect of several environmental factors on photosynthesis, Blackman found that some of them, being in the minimum, limit the influence of the other factors. This dependence of one factor on another is called "the law of limiting factors."

This law operates distinctly when at least two external factors are acting simultaneously, *e.g.*, light and the carbon dioxide content of the atmosphere. It has been pointed out that the dependence of photosynthesis on each of these two factors separately may be expressed by a logarithmic curve. Suppose, now, that the intensity of the light increases, while the content of carbon dioxide remains the same, a condition that may be observed, for instance, during the day in a greenhouse. From the moment when the intensity of light attains a level at which the carbon dioxide absorbed by the chloroplasts during a certain period is completely used up, further increase of assimilation becomes impossible, and the curve, showing the dependence of photosynthesis on the intensity of light, proceeds in a horizontal direction. If the CO_2 content of the atmosphere is increased, then the bending of the curve takes place at a higher light intensity than before, and only with a certain excess of carbon dioxide in the air may the actual dependence of photosynthesis on the intensity of light energy be clearly ascertained.

Thus it is seen that a very low concentration of carbon dioxide limits the influence of gradually increasing light. On the other hand, there are conditions where too feeble light is the factor limiting the influence of the other external conditions. Hence, the law of limiting factors may be formulated as follows: The rate or intensity of any physiological process is determined, in the first place by the magnitude of that factor, of all the external factors, which is at a minimum in proportion to demands. In other words, this factor, being in the minimum, limits the influence of all other external factors.

The law of limiting factors in its original form is of a pronounced mechanistic character, for it assumes that each factor is completely isolated and acts independently of all others. It does not take into consideration the complicated interrelations of all the factors, both with each other and with the organism upon which they act. According to this law, if one of the factors is limiting, an increase of any of the other factors will not be able to intensify a certain physiological process. Later investigations in respect to other factors and other physiological processes (Benecke, Lundegârdh, and Harder) have likewise established that the limiting action of the separate factors only weakens but does not entirely stop the influence of other factors, the controlling influence being not an absolute but only a relative one. This relationship is represented graphically in Fig. 64, showing the dependence of assimilation on the varying intensities of two factors, light and carbon dioxide content, in the aquatic moss *Fontinalis*.

The law of limiting factors clearly shows that the process of photosynthesis should be regarded as a chain of separate processes, each of which is regulated chiefly by some one of the factors of the surrounding medium. The quantity of light is reflected in the velocity of the photochemical reaction; temperature acts upon Blackman's reaction; the carbon dioxide content determines first of all the rate of its diffusion into the leaf; etc. In studying the influence of external conditions upon photosynthesis, there must always be held in view the complexity of the process as well as the possibility of direct interrelations between the separate factors. For instance, the rate of the diffusion of carbon dioxide depends not only on the concentration of CO_2, but also on temperature, etc. The water content of the

leaf and, more generally, the conditions of the water supply of the plant exert a very great and complicated influence upon photosynthesis. This influence will be discussed farther on in the chapter devoted to the water relations of plants.

The complicated influence of each of the external factors upon photosynthesis, as well as the complexity of the interrelations between the separate factors, determines the extreme complexity of the diurnal march of photosynthesis. This complexity is

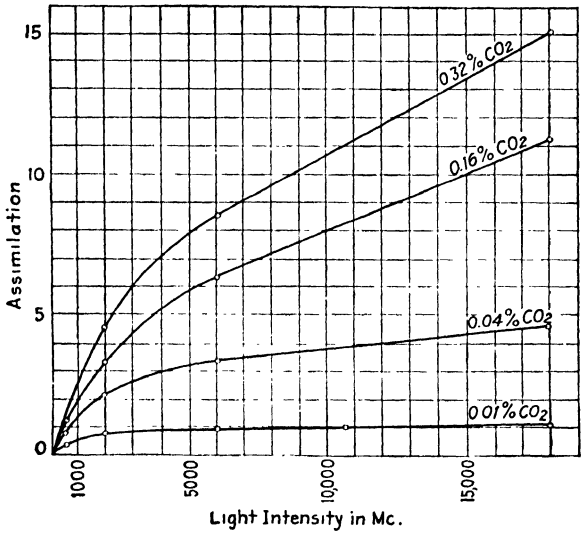


FIG. 64.—Interrelation of the intensity of light and the concentration of carbon dioxide in their influence on assimilation in the aquatic moss, *Fontinalis* (*after Harder, from Lundegårdh*).

augmented by the continuous changes in the intensity of the factors, more or less regular on clear days but most erratic on days with variable weather. That is why the diurnal course of photosynthesis is highly unstable on different days even for one and the same plant and may sharply differ on the same day for various plants.

In a moderate climate with a sufficient water supply, the general course of photosynthesis accords with the course of the sun's radiation. Beginning in the morning with the rising of the sun, photosynthesis reaches its maximum in the noonday hours and then gradually decreases toward the evening, ceasing

WEEKLY CHANGES IN WEIGHT OF CORN

Weeks after sowing	Sowing	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dry weight, in gms....	0.30	0.15	0.6	4.7	16	49	124	195	300	385	500	570	680	800	900
Increase, in gms.	....	−0.15	0.45	4.1	11	33	75	71	105	85	115	70	110	120	100
Increase, per cent.....	....	−50	300	683	234	206	153	57	53	28	30	14	19	19	12

As may be seen from the table, the weight of the plants increases continuously throughout the 15 weeks, except the 2 weeks after germination when there is a considerable loss in dry matter. The weekly increments of weight, expressed in grams, increase rapidly during the first 6 weeks, but from the seventh to the ninth week, they become more or less stationary. Very significant are the figures showing the weekly increments in percentage of the total weight of the plant. In the beginning, this relation increases very rapidly, from a loss of 50 per cent during the first 2 weeks to a maximum of 683 per cent in the fourth week. After this maximum, the percentage decreases gradually, though the absolute magnitude of the increase continues to augment. The cause of this gradual fall in the percentage of increase may be explained by the fact that during the early stages of development the plant consists almost entirely of leaves; and all the organic substance, formed during the process of photosynthesis, is consumed for the production of new leaves, which soon begin to participate in the process of photosynthesis. Later, there begins a progressive development of stems and reproductive organs, which participate little in photosynthesis but consume a large amount of assimilates for their development and in respiration.

Plants of various types show considerable differences in the general course of accumulation of dry substance. Early-ripening plants as a rule form stems and reproductive organs earlier, causing a break in the process of accumulation, and ultimately produce a lower yield than plants with more prolonged growth period. Therefore it is advantageous to sow early varieties only in regions where early frost or early drought compels the hastening of the harvest. In regions with a long vegetative period, *e.g.*, in the southeastern United States or on the steppes of North Caucasus with their warm and humid climate, it is preferable to

grow late varieties possessing a prolonged period of intense accumulation and producing higher yields.

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CHAPTER VI

ASSIMILATION OF NITROGEN BY PLANTS

50. Absorption of Nitrogen from the Soil. Nitrates and Ammonium Salts as Sources of Nitrogen.—The amount of nitrogen contained in the dry substance of a plant is rather small, as a rule not exceeding 1 to 5 per cent. Nevertheless, it plays a very important role in the life of a plant. Nitrogen is an indispensable constituent of the protein molecule, which in turn is a vital part of protoplasm. Therefore, the absorption of nitrogen is by no means a less important factor in the nutrition and growth of plants than the utilization of carbon, hydrogen, and oxygen, which form approximately 95 per cent of the dry substance.

Nitrogen is found in the environment of plants in two different forms: as elemental gaseous nitrogen, constituting almost 80 per cent of the air; and as a part of various inorganic and organic substances. Gaseous nitrogen is directly accessible to the leaves and roots of plants, since there is always a circulation of air through the soil along the capillary spaces formed between the solid particles. Insufficient penetration of air is found only in excessively dense and swampy soils. In the form of various chemical compounds, nitrogen is also present in both the air and soil. In the atmosphere, nitrogen is found as ammonia gas, which is produced by the decay of organic matter, and in the form of oxides of nitrogen formed by the combustion of nitrogen-containing substances and by electric discharges, such as occur, for instance, during a thunderstorm. The relative amount of these forms of nitrogen is quite small. A much greater variety of nitrogenous compounds is present in the soil. Here are found both the inorganic forms of nitrogen, such as the salts of ammonium and of nitric acid, and the organic compounds of incompletely decomposed tissues of plants and animals, chiefly in the form of proteins and the products of their decomposition, the amino acids.

The question as to which compound of nitrogen may serve as a source of this element for the nutrition of plants may be

settled best by means of artificial cultures. The numerous experiments, first carried out by Boussingault in the middle of the nineteenth century and frequently repeated since, have definitely shown that molecular nitrogen, N_2, cannot be utilized by the higher plants. They cannot transform into a fixed state this highly inert gas.

The compounds of nitrogen found in the atmosphere, *viz.*, ammonia gas and oxides of nitrogen, may be assimilated by plants, but their amount in general is so small that it is entirely insufficient to insure normal plant development. Practically all plants, therefore, are forced to obtain the required amount of nitrogen from the nitrogenous substances found in the soil. Hence, in the growing of plants in artificial cultures, compounds of nitrogen necessarily must be included in the nutritive mixture. Ammonium salts and nitrates are most frequently used for this purpose.

The nitrogenous substances contained in the soil may be divided into three groups: the organic compounds of nitrogen, the salts of ammonium, and nitrates. Their total amount is quite small, constituting, as a rule, not over a fraction of 1 per cent of the total dry weight of the soil. Owing to the difficulty of completely separating them from the nitrogen-free compounds, it is usually considered sufficient to determine by means of analysis the amount of nitrogen produced by the combustion of the organic substances of the soil. Hence, in reporting analytical data, the amount of organic compounds of nitrogen usually is not mentioned, but rather the total amount of nitrogen contained in these compounds. Similarly, instead of determining the weight of ammonium and nitrates, it is commonly considered sufficient to report the weight of nitrogen included in their composition. This has given rise to such abridged terms as "organic nitrogen," "nitrate nitrogen," and "ammonia nitrogen." These figures may be transferred into those showing the total weight of the corresponding substances, if it is accepted that the humus of an average soil contains approximately 5 per cent of nitrogen, potassium nitrate about 14 per cent, and ammonium sulphate about 21 per cent.

The relative quantities of the different forms of nitrogen in various soils may be seen from analyses of Russian soils made by Kossovitch. Generally it was found that the black soils ("cher-

nozem") contain 0.50 per cent of organic nitrogen, 0.002 per cent of ammonia nitrogen, and 0.003 per cent of nitrate nitrogen; the gray forest soils, respectively, 0.25, 0.001, and 0.0008 per cent of these substances; and the sandy podsol soils 0.09, 0.002, and 0.0009 per cent. These figures show that most of the soil nitrogen is found in the form of organic compounds, the inorganic ones making up but a small fraction of the total quantity present.

A further study of the question as to which compounds of nitrogen, the organic or the inorganic, are better utilized by plants, is connected with considerable experimental difficulties. Every agriculturist knows from his experience that the application of organic nitrogenous fertilizers, such as manure, markedly increases the yield. This might lead one to believe that organic compounds of nitrogen are most easily assimilated by plants. But the very fact that for fertilizer purposes it is best to use manure that is considerably decayed, suggests that the simpler compounds are better assimilated than the more complex. Experiments with soil cultures, however, so far have given no definite answer to this question. In every soil are found an enormous number of the most varied microorganisms, which decompose every organic substance introduced into a soil. The final products of this decomposition, as will be shown later, are ammonium salts and the nitrates, which are always present in soil. When an organic substance is added to the soil, therefore, it is impossible to say whether it will be utilized by the plant in an unchanged condition or whether first it will be decomposed by bacteria, and the products of its decay then absorbed by plants.

To solve this question, a method of sterile artificial cultures has been devised, in which the roots of plants are placed in a medium free from bacteria. The leaves of the plants may be left exposed to the air, as it has been established that excepting a few parasitic forms, bacteria falling upon the aerial parts of a plant from the atmosphere are unable to enter plant tissues. At the present time, there are a fairly large number of such methods of sterile culture (Maze, Shulov, Petrov, Knudson, and others). Essentially, they are but variations of those used in microbiology for the culture of bacteria, the sterile nutritive medium usually being isolated from the atmosphere by means of a layer of sterilized cotton. The stem of the germinating

seed is then passed through this cotton by means of special devices. The seed used for this purpose is thoroughly washed with disinfectants previous to germination, and then germination is allowed to proceed in a sterile medium. As a result, the roots of the plant develop beneath the cotton with a complete absence of bacteria, while its leaves spread freely in the air (Fig. 65).



FIG. 65.—Sterile water culture of a corn plant (*redrawn after Knudson*).

Experiments with sterile cultures have definitely shown that many of the organic compounds of nitrogen, such as amino acids, lecithin, etc., may be directly assimilated by plants. Their utilization, however, proceeds at a very slow rate. Plants developing in such mediums are, therefore, considerably checked in growth as compared with plants receiving nitrates or ammonium salts. Hence, it may be concluded that mineral compounds of nitrogen are better sources of this element for plants than the organic forms, and that nitrogen in humus is utilized only after it has

been transformed into inorganic form by the activity of soil bacteria. Similarly, the carbon present in humus cannot be used by plants until it is changed into the form of gaseous carbon dioxide, which is likewise produced in soils as a result of the life activity of microorganisms. Thus it is seen that a very close connection exists between the nutrition of green plants and the work of soil organisms.

The ability to construct its body from simple inorganic substances marks a distinction between a green plant and an animal, the latter absolutely requiring organic compounds for its nutrition. Green plants, therefore, may be called autotrophic organisms. They have the capacity of independent nutrition, as contrasted with heterotrophic organisms, which are nourished at the expense of organic substances produced by other living things. In the latter group are included animals as well as nongreen plants like the fungi and certain higher plants of parasitic nature, such as *Orobanche.*

The salts of ammonium and those of nitric acid are the inorganic compounds of nitrogen commonly found in soil. In water cultures, the salts of nitric acid produce the better growth. Consequently, it was believed for a long time that nitrogen was assimilated by the plant only in this form. In cases where fertilization with ammonium salts (a sulphate is most frequently used for this purpose) produced fairly good results in soil cultures, this was attributed to the fact that ammonia is first oxidized by bacteria into nitric acid, only then becoming available to the plant.

Experiments carried out with sterile cultures have shown this concept to be false. The root system of plants can absorb ammonium salts directly, and provided that they are in a sufficiently dilute solution, they cannot be considered inferior to nitrates as a source of nitrogen for plants. In higher concentrations, however, ammonium salts are toxic. They cause a weaker and more irregular growth of the roots and hence have a harmful effect on the general development of a plant.

As a general rule, it may be said that, under conditions favorable for the growth of plants, ammonium salts are assimilated just as well as nitrates. But as soon as external factors, such as temperature, illumination, and soil acidity, do not quite correspond to the requirements of the plant, the toxic properties of

the ammonium ion become manifest. Besides the direct toxicity of the ammonium ion, the harmful effect of these salts is also due to the fact that their anions are assimilated by plants in smaller proportion than the cations. They accumulate in the nutritive solution, causing its reaction to shift toward increasing acidity. Thus, for instance, if ammonium sulphate, $(NH_4)_2SO_4$, is added to the nutritive solution, it will be observed that, as the plant absorbs the ammonium, sulphuric acid accumulates in the solution, injuring the roots of the plant, checking its growth, and even causing its death. In general, salts whose cations are consumed by the plant with greater intensity than their anions are termed "physiologically acid salts." Their introduction into the nutritive solution leads to its gradual acidulation, which progresses in proportion to the growth of the plant. On the other hand, there are salts whose anions are absorbed with greater avidity than are their cations. Such, for instance, is Chile saltpeter, $NaNO_3$. Its Na^+ ion is hardly used at all by plants, while the NO_3^- anion is absorbed with great rapidity. The introduction of such a salt into a nutritive solution produces a gradually increasing alkalinity. Salts of this kind are called "physiologically alkaline salts." It goes without saying that this physiological acidity or alkalinity has no connection with the purely chemical idea of acid and basic salts.

The harmful effect of such physiological acidity in the nutritive solution may be avoided by changing the solution frequently. In soil cultures, the effect is avoided by introducing calcium carbonate (limestone or marl) into the soil, which neutralizes the free acid formed. Experiments carried out by Prianishnikov have shown that when ammonium sulphate is added to a soil simultaneously with limestone, its fertilizing effect is no less than that of nitrates.

Ammonium nitrate, NH_4NO_3, is one of the best sources of nitrogen for plants, as both of its ions are utilized at an almost equal rate. The introduction of this salt into a nutritive solution does not cause its reaction to shift in either direction.

51. The Products of Nitrogen Assimilation and Its Connection with Photosynthesis. Primary Synthesis of Protein Substances.—The plant requires nitrogen for the purpose of forming protein substances, which are an indispensable part of

the protoplasm. The proteins are the products of nitrogen assimilation. Their molecules, however, are too complex to be considered as primary products of nitrogen assimilation. Protein formation is preceded by the synthesis of some simpler compounds of the type of amino acids. Unfortunately, however, the chemistry of nitrogen assimilation is not quite so clear as is that of carbon assimilation.

The utilization of nitrates is easy to trace in plants by means of a series of color reactions. For instance, a solution of diphenylamine in concentrated sulphuric acid produces a bright blue color in the presence of the slightest traces of salts of nitric acid. From this reaction, it can be shown that nitrates are absorbed by the root hairs, and that they penetrate unchanged into the vessels of the root system and then are transferred with water in the same unaltered state through the vascular bundles of the stem into the leaves. When the plant is placed in a dark room, an accumulation of nitrates occurs in the leaves, primarily in the chlorenchyma cells. This concentration is very small, however, because the ascent of water is also considerably slower in darkness. When the plant is exposed to light, nitrates disappear from the cells of the leaf, and a simultaneous increase in protein content may be observed.

The process of nitrate assimilation thus is closely connected with that of photosynthesis. It is of importance to note that in nitric acid, nitrogen is combined with oxygen; whereas in the protein molecule, nitrogen is in the form of the amino, $-NH_2$ group, *i.e.*, in combination with hydrogen. Naturally, then, the assimilation of the nitrogen from nitrates must be followed by their reduction to an amino group. The plant being exposed to light, this reduction proceeds parallel to and simultaneously with the process essential to photosynthesis, *viz.*, the reduction of carbon dioxide.

It was at one time supposed that the reduction of nitrates was at the direct expense of light energy, and that consequently it could occur only when plants are exposed to light. Lately it has been shown that with a sufficient amount of carbohydrates present, the synthesis of proteins may take place also in darkness. This has given rise to another supposition, *viz.*, that nitrates are reduced not by radiant energy but through the action of chemical energy that is stored in the carbohydrates. Hence the role of

light in the synthesis of proteins may be an indirect one. Light is necessary for the synthesis of carbohydrates, which are consumed in the formation of protein. At any rate, light, whether directly or indirectly, plays an important part in the assimilation of nitrates; and the primary source of energy for the synthesis of nitrogenous compounds is the same chlorophyll-bearing parenchyma of the leaf where the assimilation of carbon and water elements takes place.

When ammonium salts are used for the nutrition of plants, the necessity of reducing the nitrates for the synthesis of proteins is naturally eliminated. It seems, therefore that ammonia ought to be more easily assimilated than the nitrates. But owing to its toxicity, apparently, it is not carried through the plant in an unaltered state, but is changed even in the roots into an organic substance, such as asparagine, and in this form is transferred to the leaves, where the final transformation takes place. This rather complicated mechanism seems to complicate the nutrition of plants with ammonium salts, and thus under ordinary conditions nitrates may be considered a better source of nitrogen for plants.

The formation of protein substances in the leaves from inorganic compounds of nitrogen and products of the assimilation of carbon is called the "primary synthesis of protein substances." This synthesis can be realized only in plants and is of no less significance for the existence of the animal world than is the synthesis of carbohydrate from carbon dioxide and water.

The reduction of nitrates passes through the stage of the formation of nitrites, salts of nitrous acid, oxygen being not released in a free state but utilized for the oxidation of organic compounds present in the cell. It is very probable that sugars play the role of such oxygen acceptors. The reduction of nitrates is especially favored by the formation in the process of photosynthesis of active forms of carbohydrates. This process is regulated by a special enzyme, belonging to the group of oxidoreductases.

The reaction does not stop with the formation of nitrites but continues down to ammonium, which also does not accumulate but either is transformed into asparagine or reacts directly with hydroxy acids, which arise from carbohydrates in the reduction of nitrites, and gives, with the latter, amino acids.

By means of condensation reactions, amino acids further give rise to polypeptides and finally to proteins.

All this scheme is to a considerable degree of a hypothetical character. But it has been established that during the daytime, leaves accumulate not only the direct products of photosynthesis, the carbohydrates, but likewise protein substances (Sapozhnikov, 1890), which therefore may be considered the result of photosynthetic activity. Frequently, no less than 25 per cent of the total carbon assimilated is combined in the form of protein substances. The accumulation of asparagine in leaves has also been quite clearly demonstrated. However, the leaves of plants with a very acid cell sap (sorrel, rhubarb, etc.) accumulate, instead of asparagine, the ammonium salts of organic acids, which are just as harmless to cells of these plants as asparagine (Ruhland and Wentzel, 1929). During the night, a translocation of nitrogenous substances from the leaves, chiefly in the form of amino acids, may be observed, similar to the translocation of carbohydrates. Thus, like starch, the primary protein substances represent in the leaf temporary compounds that are transformed and move to the points of utilization.

The decomposition of the protein substances formed in the leaves is brought about by means of proteolytic enzymes, belonging to the group of papainases (see Art. 14). According to Mothes (1933), this decomposition depends to a great extent on the oxidation-reduction potential. This is explained by the fact that enzymes of the type of papainases act upon proteins only in case they are activated by means of compounds containing the sulphydryl group (SH), such as cysteine or glutathione. In the presence of an excess of oxygen in general with a high oxidation-reduction potential, this group is oxidized, the enzymes lose their activator, and the decomposition of protein substances is inhibited. Conversely, with deficiency of oxygen, the enzymes are activated, and the processes of decomposition are accelerated. This is probably the reason why intensive decomposition of proteins occurs at night when stomata are closed, and photosynthesis and the liberation of oxygen, which increases the oxidation-reduction potential, are absent. In the daytime, on the contrary, synthetic processes prevail.

Besides the primary synthesis of protein substances in plants from inorganic compounds of nitrogen and nitrogen-free organic

substances, there is a continuous secondary synthesis of proteins from the products of their decomposition, the amino acids. This synthesis has already been considered in the discussion of the processes occurring during germination (Art. 19). This secondary synthesis of protein substances in plants likewise depends to a great degree on adequate provision of oxygen and ceases entirely in an oxygen-free medium. It has been established by the investigations of Zalessky (1901) and others that the dissection of massive organs, such as bulbs and potato tubers, increases considerably both their respiration and the synthesis of protein substances.

This connection between respiration and protein synthesis has been explained up to the present time by the supposition that respiration delivered the free energy necessary for synthesis. However, both hydrolysis of proteins and their inverse synthesis from amino acids are accompanied by very insignificant changes of energy supply. The participation of oxygen in the synthesis of protein substances is much more comprehensible from the viewpoint of the activation of proteolytic enzymes during reduction and their inhibition during oxidation.

52. Assimilation of Molecular Nitrogen of the Atmosphere by Soil Bacteria.—When the sources of carbon and of nitrogen are considered, it will be seen that in both instances green plants are obliged to utilize substances in a highly dilute state. The average concentration of carbon dioxide of the atmosphere is but 0.03 per cent by volume, while the average amount of nitrogen contained in soils is between 0.1 and 0.5 per cent by weight. But because of a high mobility of the atmosphere, plants have at their disposal practically all of the enormous amount of CO_2 surrounding the earth. Besides, the total amount of carbon dioxide in the air is continuously augmented not only through the respiration of animals, plants, and microorganisms, but also by the processes of combustion and by volcanic eruption. Conditions appear to be much less favorable in regard to the presence of nitrogenous substances. The amount contained in the soil is limited and is increased only through the disintegration of such plants and animals as have existed within the same regions of the earth's surface. Moreover, a constant transformation of fixed nitrogen into free molecular nitrogen takes place in nature. This happens, for instance, when nitrogenous organic matter

is burned. Likewise, in the process of denitrification of nitrates in the soil, the formation of free nitrogen in the soil usually occurs as a result of a superabundant accumulation of nitrogenous matter. If the processes resulting in the fixation of molecular nitrogen were not present, the nitrogen balance of the earth naturally would show an ever increasing deficit, and the organic world would be in danger of perishing from nitrogen starvation.

This does not occur, because among the living organisms populating the earth's surface there are many that possess the capacity of carrying out the extremely difficult synthesis of



FIG. 66.—*Clostridium pastorianum.*

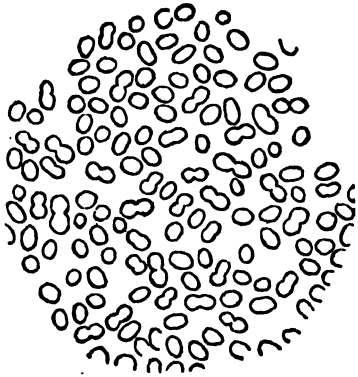


FIG. 67.—*Azotobacter chroococcum.*

organic nitrogen compounds from nonnitrogenous substances and free molecular nitrogen. These are the so-called "nitrogen-fixing" bacteria.

The majority of these nitrogen-fixing bacteria live in the soil. It has been known for a long time that fallow land becomes richer in nitrogen and that the accumulation of fixed nitrogen occurring in it is a biological process, since it is interrupted by the addition of antiseptics to the soil. Vinogradsky (1893) was the first to succeed in isolating from the soil one group of these bacteria and in studying their physiology. It proved to be a sporogenous bacillus, causing butyric acid fermentation in sugar-containing mediums and simultaneously fixing molecular nitrogen (Fig. 66). The discoverer has named it *Clostridium pastorianum.* This organism belongs to the type of anaerobic bacteria; *i.e.*, it is able to live and develop only in the absence

of oxygen and therefore always exists in soil, together with the ordinary saprophytic bacteria, which live closely associated with its colonies and consume oxygen with great avidity.

The fixation of nitrogen is an endothermic process requiring an external supply of energy. Clostridium bacteria use as a source of energy the process of butyric fermentation. Vinogradsky has found a definite quantitative relationship between the two processes. He found that 2 to 3 mg. of nitrogen are assimilated for each gram of carbohydrates fermented.

Somewhat later, the Dutch bacteriologist Beijerinck (1901) discovered another widely distributed bacterium, which he calls "Azotobacter" (Fig. 67). This organism has the capacity of very intensive respiration, which supplies it with sufficient energy for the fixation of free nitrogen. Since a much greater amount of energy may be obtained from the same quantity of carbohydrates by aerobic than by anaerobic respiration it is but natural that Azotobacter works with greater efficiency than Clostridium, being able to fix as much as 15 mg. of nitrogen per gram of sugar consumed. Besides these two most important nitrogen-fixing bacteria, there are found in the soil large numbers of others that assimilate atmospheric nitrogen. Certain authors attribute the same capacity even to some of the molds and blue-green algae, though this has yet to be confirmed. The detailed chemistry of nitrogen fixation by these organisms is not clearly understood. There are many reasons to suppose, however, that nitrogen is combined with the hydrogen of the water molecule and that ammonia is the primary product of this synthesis. Ammonia is utilized for the formation of the more complex substances, such as the amino acids and proteins.

Since the nitrogen-fixing bacteria require nonnitrogenous organic substances in order to obtain the necessary energy for their vital activities, the incorporation of plant material into soil as, for instance green manure, even if it is poor in nitrogen, considerably increases the activity of these bacteria and thus contributes to the accumulation of fixed nitrogen. A particularly intense activity is displayed by these bacteria in tropical countries, where good harvests are often secured from soils low in nitrogen and not enriched by nitrogenous fertilizers. In colder climates, however, through the activity of microorganisms there accumulates only about 10 to 20 kg. of nitrogen per hectare

yearly, which is not sufficient to restore to the soil the quantity of nitrogen, about 50 kg. per hectare, that is removed in the harvesting of crops. In order to enrich the soil with nitrogen, it is necessary, therefore, either to introduce nitrogenous fertilizers or to resort to the cultivation of leguminous plants.

53. Assimilation of Molecular Nitrogen by Leguminous Plants.—Agriculturists have noted for a long time that the growing of legumes, particularly of clover, makes the soil more fertile and subsequently increases the yield of cereals. It has

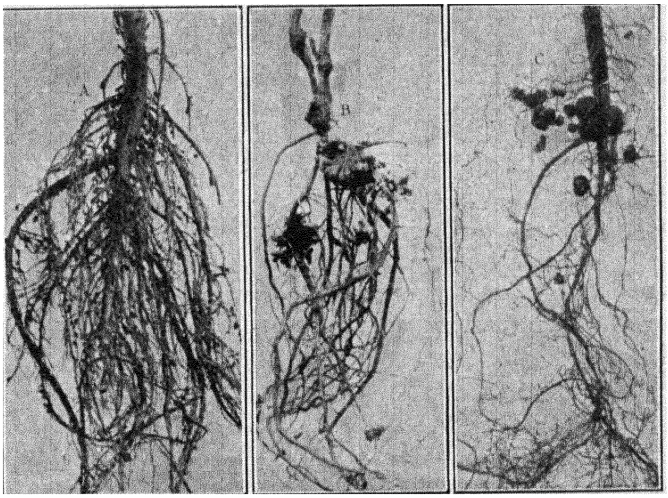


FIG. 68.—Nodules containing nitrogen-fixing bacteria on the roots of leguminous plants: *A*, red clover; *B*, sweet pea; *C*, soybean (*after Transeau*).

been established also that this augmented crop production results from an increased nitrogen content of the soil. Naturally, then, leguminous plants acquired the name of nitrogen fixers. These observations, however, proved to be contradictory to Boussingault's careful experiments, which showed that under conditions of artificial culture no higher plants, legumes included, were able to assimilate free nitrogen of the atmosphere and that their yield was wholly determined by the amount of combined nitrogen present in the nutritive solution. Hellriegel (1886) succeeded in solving this apparent contradiction. His attention was drawn to the fact that in soil certain nodules, which were absent in water and sand cultures, were formed on the roots of leguminous plants (Fig. 68). And as these nodules were found

to be filled with bacteria, it was assumed that they assimilate atmospheric nitrogen, while the plant in turn supplies these bacteria with the nonnitrogenous nutritive materials and then utilizes compounds resulting from their activity. This supposition was confirmed by direct experimental inoculation of sand cultures with small quantities of soil in which leguminous plants had been grown. Control plants, which had not been inoculated, did not form nodules on their roots and showed no evidence of assimilation of atmospheric nitrogen. Numerous nodules appeared on the roots of plants in the inoculated vessels, and the amount of nitrogen contained in the yield was much greater than that originally introduced into the soil in the form of nitrate of soda.

These tubercles found on the roots of leguminous plants are tumors formed as a result of an excessive growth of the parenchyma cells of the cortex (Fig. 69, *A*). They consist of large cells filled with bacteria. These bacteria enter the root through the root hairs (Fig. 69, *B*), whence they penetrate in threadlike formation, consisting of innumerable bacteria joined together, into the parenchyma of the root, producing a localized perforation of the cell walls and stimulating the surrounding tissues to rapid growth. The cells of the tumor become crowded with bacteria but remain alive and preserve their large nuclei. At first, the bacteria nourish themselves at the expense of the host plants. They are parasites. Hence, during the period subsequent to inoculation, the growth of the plant is somewhat retarded, but the inoculated plants soon recover and begin to show more rapid progress in their development as compared with the control plants. This occurs owing to the fact that with rapid growth of the tissues of the nodule and the increased inflow of nonnitrogenous substances from the leaves, the synthetic activity of the nodule bacteria increases, and they start to elaborate nitrogenous substances in such quantities that they suffice not only for the needs of the bacteria, but also for providing abundantly for the host plant. All the tissues of the leguminous plants are therefore rich in nitrogen, and this explains the high nutritive value of the seeds of peas, beans, lentils, and soybeans, as well as the hay of clover, alfalfa, and other leguminous plants. A part of the nitrogenous substances elaborated by the nodule bacteria diffuses from the roots into the soil and may be utilized

by the roots of other plants. It has been established by exact experiments that in sowing leguminous plants with cereals, for instance, a mixture of vetch with oats, the latter obtain considerably more nitrogenous substances than when they are sown separately. Toward the end of vegetation, the bacteria contained in the cells decrease in number and are changed into an irregular form (Fig. 69, *C*, *D*, *E*), the so-called "bacteroids." Apparently, the greater part of the bacteria die and are digested as a result of the effect produced on them by the living protoplasm of the nodular cells. The products of their disintegration

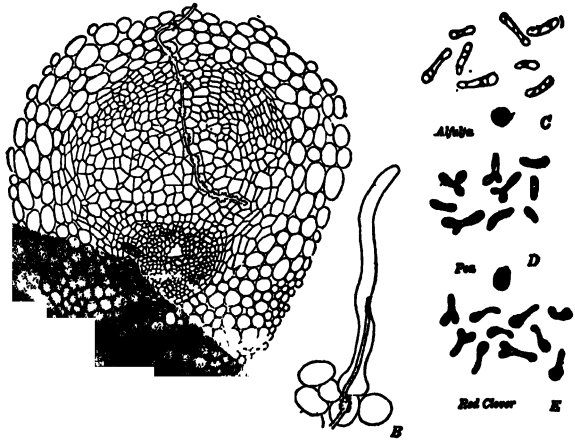


FIG. 69.—A cross section of a [illegible]odule of vetch, showing infection thread of *Bacterium radicicola*. *B*, infec[illegible] thread, having entered the root hair; *C*, *D*, *E*, bacteroids from different plants (*after Smith, et al.*).

are absorbed and assimilated by the plant. The nodules having rotted and separated from the roots, the surviving bacteria are liberated into the soil where they continue to exist, though here they multiply more slowly than in the nodules. With a new seeding of a leguminous plant in the same soil, its roots are again inoculated with the bacteria.

The relationship existing between leguminous plants and nodular bacteria is usually regarded as symbiosis, or the intimate union of two organisms, both of which are benefited. The bacteria receive carbohydrates from the plant. In turn, the bacteria supply the plant with combined nitrogen, which they elaborate from the inexhaustible source of this element, the atmosphere. This relationship, like other types of symbiosis,

however, may be regarded as a sort of balanced parasitism. First, the bacteria are the assaulting party, behaving like parasites in respect to the plant and causing a temporary delay in development and even, in some instances when the plant is not sufficiently strong, its death. Later, however, the upper hand is gained by the plant, which then absorbs from the tubercles the nitrogen compounds produced by the bacteria and finally digests the greater part of them.

The bacteria found in nodules have been isolated in pure cultures and have been named *Bacterium radicicola*. Several races of such bacteria have been isolated, each of them corresponding to a definite group of leguminous plants, whose roots they can inoculate. The bacteria from beans readily inoculate vetches but are ineffective on alfalfa. In introducing new leguminous plants, such as, for example, soybeans or lupines is therefore necessary to incorporate into the soil artificial cultures of such nodular bacteria as are adapted to these plants (Fig. 70). A proper "bacterial inoculation," therefore, often increases the yield considerably. The rather widely used preparation "nitragin" is a culture of nodule bacteria. Inoculation with these bacteria may be found especially necessary when [illegible] are grown on drained swamps, because these are usually absent in boggy soils. All leguminous plants have the capacity of entering into symbiosis with bacteria, this being an important physiological peculiarity of this group of plants. It is rarely found in other plants. Among the trees are found similar, but perennial, tubercles on the roots of the alder *Alnus sp.* and *Eleagnus;* while [illegible] trees and shrubs, belonging to the family of [illegible] bacterial tubercles are on the leaves. In these

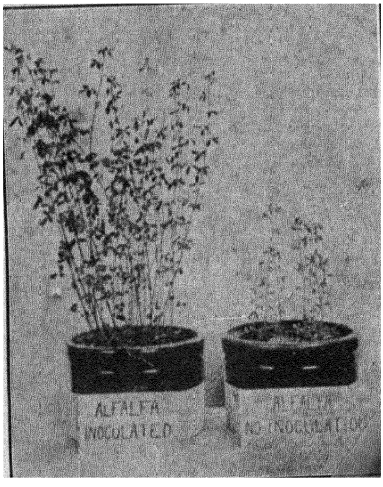


FIG. 70.—Alfalfa grown in soil poor in nitrogen: left, inoculated with specific bacteria; right, not inoculated. (*Smith, et al.*).

cases, *Bacterium radicicola* is not present, but other similar microorganisms are found. The physiological peculiarities that prevent the nitrogen-fixing bacteria from living on the roots of other plants are not understood as yet. Evidently there is in this case the same specific adaptability of organisms toward each other that is observed in most cases of real parasitism.

54. The Nitrogen Cycle in Nature. Artificial Nitrogenous Fertilizers.—The ability of nodule bacteria in leguminous plants to fix molecular nitrogen of the atmosphere is of enormous importance in the economy of nature in general and in the economy of agriculture in particular. Leguminous plants are widely distributed over the earth's surface. They are quite common in every plant association. The nitrogen that they accumulate is of such an amount that it is not only sufficient for their own nourishment and for abundant storage in their seeds, which are especially rich in nitrogen, but it also produces a general increase in the nitrogen content of the soil. This enrichment of the soil is due to rotting of fallen leaves, of nodules remaining in the soil, and of other disintegrating parts of the plant.

The capacity of leguminous plants to accumulate nitrogen in the soil explains their popularity in agricultural practice. The favorable effect of their cultivation on the subsequent yields of cereals was known long before Hellriegel's time. One of the classical experiments carried out by Laws and Gilbert at one of the first agricultural experiment stations in Europe, at Rothamstead, England, has shown that when wheat alone was grown for 10 successive years on one of two identical fields and wheat in succession with leguminous plants on the other, 5 yields of wheat from the second plot were equal to 10 from the first. In addition, 5 harvests of leguminous plants had been gathered from the second plot, and each of these contained more nitrogen than did the 5 crops of wheat. Thus, the effect produced by leguminous plants seems to be almost miraculous. Not only are the plants themselves well nourished, but in addition the most important nutritive element is left in the soil in greater abundance than it was contained before the experiment.

In general, plant production may be considered as a practice in which highly valuable food and technical products are created from raw material, like carbon dioxide, water, and radiant energy, which cost practically nothing. The requisite amount of

nitrogen and mineral substances, however, is often quite expensive. Hence, the struggle for the possession of the most fertile territories of the earth is in its essence a struggle for fixed nitrogen, which most frequently is the limiting factor in plant production. The growing of legumes is a means of utilizing a free source of a very valuable substance, the molecular nitrogen of the atmosphere.

Because of the abundance of nitrogen in all parts of leguminous plants, it is good agricultural practice to sow some leguminous plant on soils poor in nitrogen. When the plants have developed sufficiently, they are plowed into the soil to produce green manure. Cereals sown after this utilize the nitrogen that has been fixed and accumulated by the leguminous plants.

Owing to the ever increasing intensification of agriculture, however, the beneficial effects of leguminous plants, as well as that of free-living soil bacteria, have been found to be insufficient, and other more rapid ways of enriching soils with nitrogen must be discovered. Until quite recently, almost the only method of achieving this was by the application of Chilean nitrate of soda, enormous deposits of which in South America have been used commercially for many years.

The introduction into the soil of such organic material as manure, animal residues from slaughterhouses, city refuse, and the like, is nothing but an incomplete return to the earth of what was previously taken from it, as part of the nitrogen always is lost in the processes of decay and denitrification or is carried through leaching to the ocean by rivers.

A desirable feature of potassium nitrate, when compared with stable manure, is its more concentrated form. One ton (1,000 kg.) of stable manure contains, on an average, only about 4 kg. of nitrogen. In order to obtain the same amount of nitrogen, 20 kg. of potassium nitrate are sufficient, or only one-fiftieth of the weight. That is why saltpeter, $NaNO_3$, may be profitably transported great distances, and many countries in Europe and elsewhere are provided with it from Chili. On the other hand, stable manure contains other nutritive substances necessary for the plant. Moreover, its application considerably improves the physical properties of the soil. Fertilization with stable manure is, therefore, one of the chief methods of improving soils poor in

nitrogen, even when the so-called "mineral fertilizers" are extensively used.

The deposits of saltpeter, however, are being gradually exhausted. This seems to threaten mankind with the possibility of nitrogen starvation. Modern industry, however, has come to the aid of agriculture. Several years previous to the World War, a method of obtaining nitric acid from the air by means of a high-potential electric discharge was found. This method is being widely applied, especially in Norway, a country rich in waterfalls, which produce cheap electric power. Hence the product, calcium nitrate, bears the name of "Norwegian saltpeter." During the World War, the German chemist Haber discovered another method of fixing atmospheric nitrogen by combining it with hydrogen, thus forming synthetic ammonium. At the present time, there are in operation in Europe and elsewhere several factories that produce hundreds of thousands and even millions of tons of synthetic ammonium and nitrate salts. It is very likely therefore that in the near future the requirements of agriculture will be fully satisfied by artificial nitrogenous fertilizers produced from the air. Then the increase of yields will not depend, as heretofore, on the growing of nitrogen-fixing leguminous plants.

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CHAPTER VII

ABSORPTION OF MINERAL ELEMENTS BY PLANTS

55. Mineral or Ash Constituents of the Plant. Their Composition and Origin. Indispensable and Supplementary Mineral Elements. The Significance of Mineral Elements.—One of the principal methods of analysis of the plant consists in burning it. During this process, the carbon is determined as carbon dioxide, hydrogen, and oxygen in the form of water, and nitrogen in the form of molecular N_2. After the burning of the dry substance of a plant, there always remains a nonvolatile residue, the ash. This, too, represents an indispensable part of the living substance.

The quantity of ash in different parts of a plant is variable. The least amount, about 1 per cent, is found in the wood of trees, which consists primarily of the walls of dead cells. Seeds contain about 3 per cent of ash; the main vegetative organs, stems, and roots of grassy plants, 4 to 5 per cent; while the leaves may have as much as 10 to 15 per cent. It is of interest to note that a relatively high concentration of ash, approximately 7 per cent, is found in the bark of woody plants. Tissues consisting mainly of living cells are usually richest in ash. It must be understood that these are approximate figures. They will vary not only with different plants; even within the same organs of a species, the quantity of ash can fluctuate considerably. Leaves of potatoes, for instance, may contain from 5 to 13 per cent of ash; those of beets, 11 to 21 per cent; and of rape, 8 to 15 per cent. The ash content depends largely on the composition and moisture content of the soil. In general, one may say that the richer in salts the soil is and the dryer the climate, the more ash accumulates in the plant, although a direct proportionality does not always exist.

The composition of the ash is very complex and variable. Almost all of the elements, including the rarest ones, have been found in the ash of some plant. Many of the rare elements are dispersed in the crust of the earth in such small amounts that they may not be discovered by ordinary analysis, but they may be

present in a considerable concentration in plants. Bromine and iodine, for example, accumulate in large quantities in the seaweeds. Some geologists, as for instance Vernadsky, attribute to the accumulating power of living organisms a significant role in the general circulation of the rare elements on the earth's surface.

The following elements are most often found in the ash of plants: K, Na, Ca, Mg, Fe, Mn, Zn, B, Al, Si, P, S, and Cl. Their presence does not mean, however, that all of them are really necessary.

Analysis alone proves to be insufficient for solving the question of which of these elements is really indispensable. The problem requires the application of synthesis, and the basic synthetic method employed for this purpose is the method of artificial cultures.

According to this method, instead of using natural soil, which is of a very indefinite composition difficult to control, the plants are grown in an artificial medium, all the constituent parts of which are exactly determined. Well-washed pure quartz sand, fine beads, and even scrap platinum may serve as such an artificial substrate, but the most convenient is the method of water cultures. In this case, the roots remain, not in soil, but in water in which are dissolved all the elements that the plant cannot obtain from the atmosphere but that nevertheless are absolutely indispensable to its development. By the use of culture solutions, it is possible to know the exact concentration of each ion or substance.

Experiments first carried out in the fifties of the last century, and since then many times repeated, have shown quite definitely that a very limited number of elements must be introduced into the solution for the normal development of the plants. One of the most commonly used solutions for water cultures, *viz.*, Knop's solution, contains the following salts: $Ca(NO_3)_2$, 2 to 4 parts; KH_2PO_4, 1 part; $MgSO_4$, 1 part; KCl, ½ part; and a small quantity of $FePO_4$ dissolved in 4,000 parts of water. More recently, Shive and Tottingham have used a solution composed of only three salts, *viz.*, KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ with the addition of traces of an iron salt. With sufficient care, plants develop on such solutions as well as on the most fertile soils and give excellent yields (Fig. 71). If but one of the ele-

ments of the salts used for these solutions is excluded, be it K, N, or Mg, the plant will not develop; or more exactly it will develop so long as the reserves in the seed allow. Thus it can be said that normally the plant must obtain all these elements from the soil. Carbon alone is not among these elements, in spite of the fact that carbon constitutes almost half of the weight of the dry substance of every plant. Hence it may be concluded that the plant does not obtain its carbon from the soil and does not require for its development carbon-containing constituents of the soil, such as soil carbonates.

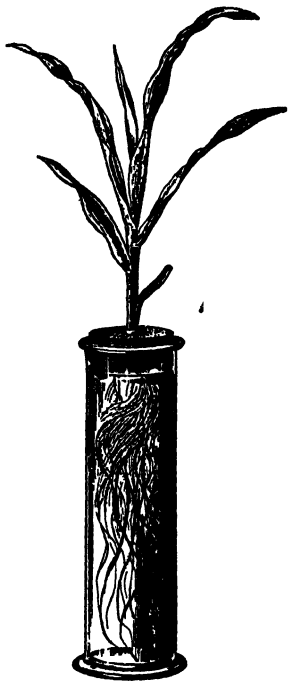


FIG. 71.— A water culture (*after Nathansohn*).

On the contrary, it is not difficult to ascertain that the carbon of the carbon dioxide present in the atmosphere although in quite insignificant amounts, forming only about 0.03 per cent of its volume, still is absolutely indispensable for the nutrition of plants. To show this, place a plant in an atmosphere deprived of carbon dioxide, for instance, under a glass bell jar, into which the air enters through a layer of soda lime, which absorbs carbon dioxide (Fig. 72). A check in the further accumulation of substance will very soon be observed, and the plant will soon show symptoms of starvation. The same effect will be obtained if the roots are immersed in a solution deprived of carbon constituents, or if they remain in a soil rich in humus. In the latter case, special care must be taken to prevent the penetration of carbon dioxide from the soil, where it is continuously being produced by bacterial activity, into the glass bell jar covering the aerial parts of the plant. For this purpose, a container holding a solution of potassium hydroxide may be placed so as to absorb any carbon dioxide that might diffuse out of the soil.

Both water cultures and experiments with the growth of plants in an atmosphere deprived of carbon dioxide show that the need of the plant for carbon is entirely satisfied by the carbon dioxide

The indispensable ash elements may be divided into two distinct groups: the nonmetals, such as sulphur and phosphorus; and the metals, potassium, calcium, magnesium, and iron. Sulphur and phosphorus are found combined with the most important organic substances of the protoplasm in the proteins and nucleoproteins and should therefore be referred to the group of organogens, like the four elements that are volatile on burning (C, N, H, and O). As they possess a number of similar prop-

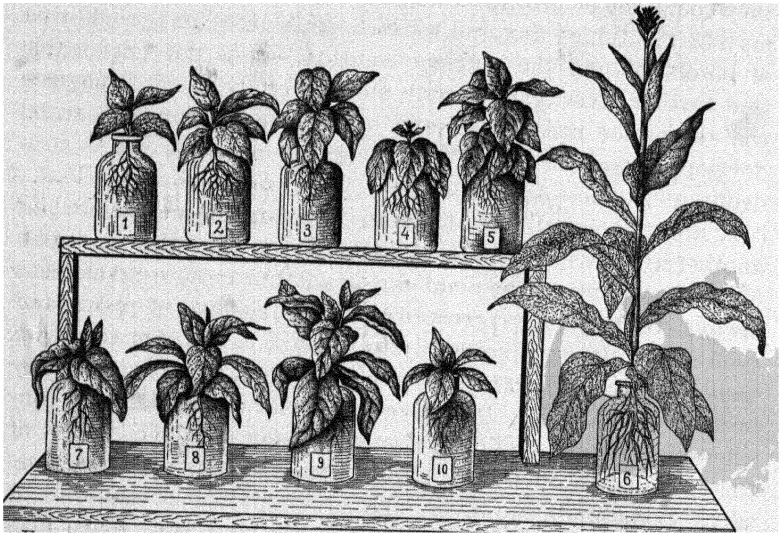


FIG. 73.—Water culture of tobacco in nutrient solution and with deficiencies of various elements. 1, without N; 2, without P; 3, without K; 4, without Ca; 5, without Mg; 6, normal nutrient solution; 7, without B; 8, without S; 9, without Mn; 10, without Fe.

erties, S and P may be compared with nitrogen, which is also indispensable for the building of the protein molecule. The metals, on the contrary, are present in the plant mostly as free ions. This is especially the case with potassium, up to 98 per cent of which is found in the plant in ionic form and may be extracted from the dry plant substance by water, combustion not being necessary. Metallic elements acting upon the colloidal substance of the cell play the role of regulators of vital processes stimulating certain of them and inhibiting others.

This division, however, is not quite distinct. Some organic compounds of metals are present in the most important parts of

the cell, where they have a very vital function. Magnesium, for instance, is found in the chlorophyll molecule. Hence, at least some of the metals may be considered nutrient substances. Phosphorus, as phosphoric acid, may be found in both inorganic and organic form. In leaves, up to 50 per cent of the phosphorus is found in the form of phosphates, and during the germination of the seeds an almost complete disintegration of all organic phosphorus compounds takes place. The separation of the ash elements into two main groups as nutrient substances and as regulators of vital processes, however, accords in general with their role in the life of the plant. It must not be forgotten that the same element in some combinations may be considered as belonging to one group; in other combinations, it may be in the other group.

53. Sulphur and Phosphorus, Their Metabolism in Plants and Their Cycle in Nature.—Sulphur and phosphorus are just as important to the plant as is nitrogen. Both of these elements enter into the composition of protein substances, sulphur being found in all proteins, phosphorus only in the more complex ones, in the nucleoproteins. It is also present in the other complex proteins of the protoplasm that have been studied but little and often are known by the general name of "plastins." As the nucleoproteins and the plastins form vital parts of the living cytoplasm and nucleus, it is quite clear that sulphur and phosphorus are necessary for the plant.

As has already been seen in examining respiration, compounds of sulphur and phosphorus play a significant part also in the performance of this most important process. In the beginning of the oxidative disintegration of sugars, phosphoric acid combines with them giving hexosephosphate, which is subjected to further transformations with the participation of respiratory enzymes, while the compounds containing the SH group, such as cysteine and glutathione, participate most actively in the oxidation-reduction processes taking place during respiration. Many authors explain the well-known highly toxic action of the cations of heavy metals, such as mercury and silver, by their capacity of replacing hydrogen in the sulphydryl group and thus excluding it from the cycle of processes indispensable for respiration.

Of the numerous compounds of sulphur, higher plants absorb only the sulphate ion, the most important source being various

sulphates, including the slightly soluble calcium sulphate, gypsum. The free sulphates enter the roots without undergoing any change. They ascend with the water stream through the vessels, reach the chlorophyll-bearing cells of the leaf, and here, together with nitrogen and the carbohydrate products of photosynthesis, are used in the building of protein substances. At the same time, reduction processes take place; for in the protein molecule, sulphur is combined with carbon and hydrogen, not with oxygen. The mechanism of the reduction of sulphur is not understood as yet.

When plant or animal tissues decay in the soil, sulphur is split from the protein molecule in the form of hydrogen sulphide, just as nitrogen is set free in the form of ammonia. But, unlike ammonia, hydrogen sulphide is not available to plants, being very toxic to the roots. It may become available, however, when oxidized to sulphuric acid through the activity of special bacteria, which have already been mentioned in Art. 42.

Like sulphur, phosphorus is available to plants only in the highest form of oxidation as salts of orthophosphoric acid. It does not undergo quite such complex transformations in the plant body, however; for it is found in the same oxidized state in the proteins, which in this respect may be regarded as substitution products of phosphoric acids. Various esters of phosphoric acid are found in plants. Some of these may represent the reserve products of phosphorus, *e.g.*, phytin, $C_6H_6(OH_2PO_3)_6$, which is an ester of the cyclic hexatomic alcohol inositol and contains 6 molecules of phosphoric acid. Like many other plant reserve products, phytin is digestible by animals. Hence it is widely used in the diet as a remedy in cases of phosphorus starvation. Another group of phosphoric acid compounds is represented by the phosphatides. Like the proteins, they form indispensable compounds of the protoplasm. Moreover, they seem to play an important role in determining the osmotic properties of the cell, as has already been discussed in Art. 7.

A considerable part of the phosphoric acid, sometimes 50 per cent of the whole amount, remains in the plant in ionic form and plays a very important role in the regulation of the active concentration of hydrogen ions in the plant (see Art. 62). Many inorganic phosphates are found in those parts of the plant where

an energetic mobilization of the reserve substances takes place, for instance, in germinating seeds.

When plants or animals decay, the phosphoric acid is liberated largely in the form of inorganic salts, which are available to plants. Complex organic compounds of phosphorus can be utilized only with great difficulty. This has been proved by numerous sterile cultures of plants that included such substances. If the cultures were successful, *i.e.*, if they remained sterile to the end, the plants developed very poorly. If by accident they were infected by bacteria that caused a breakdown of the complex phosphorus compounds, then the plants grew much better (Shulov).

57. General Role of Cations and Their Specific Activity.—The role of the cations in the life of the plant is more complex and less understood than that of the anions of phosphoric and sulphuric acids, which have been discussed. Usually cations are found in plants in the uncombined condition. Nevertheless it has been established by artificial cultures that a certain combination of cations is quite necessary to the normal development of a plant and that none of them, neither potassium, magnesium, calcium, nor iron, can be excluded from the nutrient solution or replaced by another element. Thus each of them must have some specific function.

The great advances that are being made in the study of colloidal chemistry begin to reveal the physiological significance of the cations, though this subject is still far from being completely known. As has already been seen, the colloidal state represents a condition of unstable equilibrium. Colloids may be regarded as heterogenous systems of fine suspensions of solids in water or of emulsions of small droplets of oil.

Of the forces that hold the colloidal particles suspended in the liquid, preventing their precipitation and their adherence to each other, the electric charge of the particles is of first significance. All of them carry the same electric charge, while the suspending liquid is of the opposite charge. Hence their mutual repulsion. When an electrolyte is introduced into a colloidal solution, the electrostatic equilibrium is immediately changed. There results either a diminished or an increased charge of the particles, which causes their precipitation, or an increased stability of the colloidal suspension (see Art. 2).

All vital processes of the cell, such as the movement of protoplasm, its increase in mass during nutrition, and the growth of the cell, are dependent on changes in the stability of the colloidal system. Cell colloids may change from sols to gels and vice versa, as for instance during the growth of cell walls, the formation of starch grains, or the division of chromosomes. Thus the electrolytes may be considered most important regulators of many functions of the cell. The rate and even the direction of various processes of the cell may be influenced by the composition and concentration of the salts. Since the effect of the ions on the colloids depends on their electric charge and specific properties, one should not be surprised that the presence of an intricate system of anions and cations is necessary for the complex colloidal-chemical phenomena that take place in the cell.

The requirements of the cell for a definite combination of cations make it very difficult to study the specific role of each of them. If one element or another is excluded from the nutrient solution, it can only be ascertained that the development of the plant has been inhibited. In an experiment with buckwheat, for instance, the following results were obtained: In a complete nutrient solution, the weight of the plant was 13.8 times greater than that of the seed; in a solution without K, 9.2 times greater; in one without Ca, 1.3 times; without Mg, 5.1 times; and without Fe, 7.3 times. It would be a mistake to conclude from these results that the elements, the exclusion of which gave a small decrease in yield (K, Fe), are less necessary than the others. Two contributing causes may have influenced the visible effects. There may have been a relatively greater storage of these elements in the seed, or else their absence may have caused less evident disorders in the organism. In general, it must be admitted that very little is definitely known of the specific functions of the different cations and of the various disturbances caused by their absence. The only fact established with certainty is that each of the cations has its role, which cannot be taken by another, even one closely related in the periodic system. Thus potassium cannot be replaced either by lithium or by sodium, and only a few fungi allow a partial substitution of rubidium for potassium. Calcium, likewise, cannot be replaced by any of the alkaline earth elements, and iron cannot be changed for either nickel or cobalt.

The role of the various metals in the life of the plant will be discussed only in general. Potassium is closely connected with the vital activity of protoplasm, since most of it is found in the meristem and in general in young organs, whose cells are rich in protoplasm. An important role is ascribed to potassium in the general metabolism of the cell, especially in the formation of carbohydrates and proteins. In the active regions of the plant, as much as 50 per cent of K_2O may be found in the ash. Of all the necessary cations, potassium is the only radioactive element. Consequently, it may also have some other significance for the plant as yet unexplained.

The magnesium content of the ash is considerably less than that of potassium. Both seem to be widely distributed in the various organs. Like potassium, magnesium is found mainly in the seeds and young organs, where it constitutes 10 to 15 per cent of the total ash content. Its general physiological function evidently is similar to that of potassium. Besides, magnesium is present in some of the most important metalorganic compounds, of which chlorophyll is a conspicuous example. Up to 50 per cent of magnesium may be found in the combined state, while potassium is present in the plant almost entirely in the form of free ions.

Regarding the demands for calcium by plants, one must note that for the lower organisms without chlorophyll, such as the fungi and bacteria which in general have the same requirements for cations as the higher plants, calcium is not absolutely necessary. Calcium is abundant in the older parts of higher green plants, for instance, in the bark of trees, and in old leaves. One of its most important functions seems to be the neutralization of poisonous oxalic acid, which is formed in many plants as a by-product of metabolism. In many plant tissues, crystals of calcium oxalate occur as druses, raphides, and other formations. A small amount of calcium is, however, absolutely necessary for the growth of young tissues; and if calcium is excluded from the nutritive solution in water cultures, a marked reduction of the root system is observed. A sufficient amount of calcium seems to be very necessary also for proper growth and development of legumes.

Iron is required for the formation of chlorophyll. In its absence, the plants become chlorotic. This is not the only role

of iron in the life of the plant, however, as it is equally necessary for organisms without chlorophyll. As has been seen in Art. 24, Warburg attributes to iron a central role in the process of respiration and supposes likewise that it participates in photosynthesis.

The significance for the plant of those slight traces of zinc, boron, silicon, and perhaps some other elements, the necessity of which for the successful growth of plants in water cultures has been recently revealed, remains unknown so far. Evidently these ions have a role as catalysts necessary for certain processes of metabolism.

The significance of separate cations, such as potassium, as well as of anions, especially phosphoric acid, for blooming and fruiting will be discussed later in Chap. XII, devoted to the problems of production.

58. Absorption of Mineral Elements by the Roots.—For a long time, the absorption of mineral elements by the roots of plants was regarded as a simple process of their diffusion from the soil. Especially great significance was attributed to the so-called "transpiration stream," *i.e.*, the movement of water from the soil into the roots and upward along the stem to the leaves, caused by the continuous evaporation of water from the leaves in transpiration (see Art. 68). According to this concept, the highly dilute soil solution penetrates into the roots in an almost unaltered state, ascends the stem, and is then concentrated in the leaves as a result of the evaporation of water. The process of transpiration was regarded as one of the essential conditions of mineral nutrition of plants.

Further investigations have shown, however, that the process is considerably more complicated and that the amount of mineral substances penetrating into and accumulated within the plant is not proportional to the amount of water that has passed through the plant. The experiments carried out by Hasselbring on the Cuban tobacco plantations are especially interesting in this respect. In the growing of cigar tobacco, large areas are shaded by means of cheesecloth. This greatly decreases the general amount of water used by the plants, but the accumulation of mineral substances proves to be not less but even greater in the shaded plants, as compared with those grown in full sunlight.

It is convenient to compare the absorption of water and mineral salts by plants grown in water cultures. Numerous

investigations by various authors have shown that the salt solution almost never penetrates into the roots in the same concentration that is present in the nutrient solution. In very dilute solutions, the plant absorbs salts in excess of water, and the solution gradually decreases in concentration. Conversely, in concentrated solutions the plant absorbs more water than salts, and consequently the solution becomes more and more concentrated. The concentration of the nutrient solution has to be selected according to the needs of the plants as well as in relation to the conditions of water loss.

The independence of the absorption of salts and water is most convincingly proved by the well-established fact that various salts are absorbed by plants at a different rate and that even the anion and cation of one and the same salt penetrate in unequal proportions. This phenomenon has already been encountered in the discussion of the nutritive value of ammonium and nitrates, when there were distinguished physiologically acid salts, whose cations are absorbed more rapidly than the anions, the solution consequently becoming acid, from the physiologically alkaline salts, in which, conversely, the anion is absorbed more readily than the cation.

This difference in the absorption of separate anions and cations leads to a gradual alteration of the nutrient solution originally presented to the plant roots and very often in a direction unfavorable to the plant. In the growing of plants in water cultures, it is necessary, therefore, to change the nutrient solution from time to time, usually every 10 to 15 days.

Knop's is one of the best nutrient solutions; in his solution, these alterations in composition are small. Prianishnikov has recently given much attention to this side of the question, and the nutrient solution prepared by one of his coworkers, Cincadze, is being widely used in experiments with water cultures. An adequate composition of the nutrient solution is of special importance in the growing of plants in sand cultures, where it is not so easy to change the solution completely.

The absorption of mineral substances by the roots of plants is a very complex process. Its first stage consists in the penetration of the salts from the soil solution into the root hairs and the surface cells of the root in general. This penetration is controlled by the same laws that have already been discussed

in connection with the intake of dissolved substances into plant cells in general (see Art. 8 in Chap. I). A great significance in this process may be attributed to the specific permeability of these cells to definite ions, to Donnan's equilibrium connected with the presence of colloids in the cell, and to the cells' active absorption of ions by the expenditure of the energy obtained by respiration.

During recent years, the attention of investigators has been especially attracted to the connection existing between absorption of ions and respiration of roots. The investigations of Hoagland, Petri, Lundegårdh, Sabinin, and others have definitely established that respiration of the roots is one of the essential conditions for the absorption of salts; and this explains the favorable influence, observed long ago, of the aeration of soil and the bubbling of air through water cultures for the growth of plants.

The nature of this connection between respiration and absorption of ions is not clear as yet, and various authors think it occurs in different ways. The main role evidently belongs to the function of H^+ and HCO_3^- ions, formed in respiration and translocated to the surface layers of the protoplasm, to exchange with the cations and anions present in the surrounding solution. The intensity of respiration is determined to a considerable degree by the accumulation of respiratory materials, the carbohydrates. This accumulation in turn depends on the intensity of photosynthesis, which is the chief source of carbohydrates in the plant organism. Investigations establish quite definitely a close connection between the accumulation of organic substance and mineral salts in the plant. This connection was frequently noted by previous investigators, but its nature remained obscure.

The mutual interdependence of the main vital processes in the plant, *viz.*, photosynthesis, respiration, and absorption of mineral salts, may serve as a good example of the importance of studying the various processes occurring in the plant, not isolated from the rest of the functions and from the life of the organism as a whole, but in close mutual connection and interdependence.

59. Mineral Nutrition of Plants Grown in Soils.—In soil, the mineral nutrition is much more complicated than in an artificial culture; for the plant encounters here a great variety of com-

pounds of different elements, which are continually reacting with each other. Only a small part of the mineral salts found in the soil are present in water-soluble form and directly available to the plant. The greater part are either adsorbed by the colloidal particle or else remain in a solid state as water-insoluble minerals or organic substances.

It has been known for some time that the water solution obtained from even the most fertile soils contains such minute quantities of nutrient salts that it cannot produce a normal development in plants. This can be easily proved by growing plants in a solution leached from a layer of soil. From this it is clear that plants must be able to feed upon adsorbed and insoluble mineral substances.

The property of the soil to adsorb and to retain firmly dissolved substances has been termed its "adsorbing capacity," and the colloidal part of the soil determining this capacity is called the "soil-adsorbing complex." The phenomenon of the absorption of different substances by the soil, especially of cations, has been studied in detail by Gedroitz, whose classical work threw more light on this question, which was previously in a very confused state. Gedroitz distinguishes five various absorbing capacities: (1) mechanical, (2) physical, (3) physicochemical, (4) chemical, and (5) biological. The most important is the physicochemical adsorbing capacity, otherwise termed by Gedroitz the "exchanging capacity." The phenomena of base exchange in soils have been much clarified by recent American investigations.

The mechanical absorbing capacity is shown by soils just as by any porous body. This is the simple filtering out of fine particles held suspended in the water when it is being percolated through the soil. Physical adsorption is the result of the action of the force of surface tension at the interface between the solid phase and the soil solution. This, if positive, causes an increase of the concentration of the dissolved substances near the surface of the solid soil particles. This phenomenon is termed "adsorption" and is widely used in chemical technology, for instance, in purifying sugar solutions by means of animal charcoal. The magnitude of physical adsorption is dependent upon the size of soil particles. The finer the particles, the larger the total surface and the greater the adsorption.

Such a concentration on the surface of solid particles of soil is shown by molecules of electrolytes dissolved in the soil solution. Physical adsorption is the cause of the lack of uniformity of the soil solution, which is more concentrated near the surface of the soil particles and becomes more diluted farther away from them. Some substances show a negative adsorption. Their particles are not attracted but on the contrary are repelled by the soil particle. Some of the anions, *e.g.*, chloride and nitrate ions, behave in this way, and therefore they are easily filtered through the soil, not being retained by it.

The physicochemical base-exchange capacity of the soil is of major importance for the nutrition of plants. It is the capacity of the soil to exchange a part of the cations retained on its solid particles for an equivalent amount of cations present in the surrounding soil solution. The cations adsorbed disappear from the solution and are deposited on the solid particles of the soil. An equivalent amount of other cations passes from the soil particle into the solution. The organic substances of the soil, such as humus, represent adsorption complexes of organic colloids, products of the decay of plant and animal residues with various cations, chiefly Ca and Mg. The inorganic, or "zeolite," part of the soil was found by Gedroitz to be a complex alumino-silicate aggregate capable of exchanging its bases for the bases of salt solutions. Both the inorganic and organic particles participate in base exchange. The cations, or bases, present in the adsorbing complex of the soil that are capable of base exchange are known as the "exchange bases." Calcium and magnesium in most cases are exchangeable, as well as sodium in alkali soils and hydrogen in acid soils. The adsorptive force of the soil particles is great, and exchangeable bases can be extracted from the soil only by means of displacement by other cations.

Chemical binding of substances by the soil is observed in cases where the substance introduced into the soil reacts with soil compounds to give insoluble products, which precipitate and thus associate with the solid phases of the soil. Such phenomena are observed, for instance, in introducing phosphates into a soil that is rich in calcium salts, when an insoluble tricalcium phosphate, $Ca_3(PO_4)_2$, is formed. Chemical binding chiefly determines the holding of phosphates and in a lesser

degree of sulphates. This has a great significance for the fertilization of soils with these salts.

Biological absorption is the result of the vital activity of microorganisms, bacteria, fungi, and others, which populate the soil. In absorbing cations and anions necessary for their nutrition, these organisms remove them from the sphere of soil reactions and thus contribute to the fixation of various compounds, which return into general rotation only after the death of the organisms and the disintegration of their cells in the processes of decay. As a matter of fact, the absorption of soil constituents by the roots of higher plants themselves may be considered as biological absorption.

The adsorption capacities of soils, especially those of physicochemical and physical nature are of great importance for the nutrition of plants with mineral elements. Owing to this phenomenon, potassium and ammonium fertilizers introduced into the soil are retained by it and their leaching from the soil is prevented. At the same time, they remain available to the plant, for they may be easily displaced from the adsorbing complex by means of base-exchange reactions. Physicochemical together with physical adsorption prevents an excessive increase in the concentration of the soil solution, for instance, during drying of the soil or after the introduction of fertilizers, for it transfers a part of the cations into an adsorbed state. It regulates the chemical composition of the soil solution, providing it with bivalent ions that balance the action of the univalent Na^+ and K^+ ions. As a matter of fact, the whole process of the absorption of salts from the soil by plant roots to a considerable degree represents exchange reactions between the root cells and the soil-adsorbing complex through the medium of the soil solution.

Since close contact between the absorbing and the adsorbing surfaces contributes greatly to facilitate the exchange reactions, root hairs usually adhere very closely to soil particles (Fig. 74). That is why when young rootlets densely covered with root hairs hold a coating of soil particles, it is almost impossible to wash these away completely without injuring the tissues (Fig. 75).

Such a close contact of the root hairs with soil particles has still another significance. Not only can the roots utilize solutes and adsorbed substances, but they also are able to dissolve

substances that are insoluble or slightly soluble in water. This function of the roots was discovered by Sachs in a very simple experiment, which has since been adapted for demon-

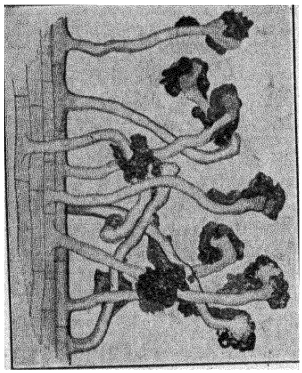


FIG. 74.—Attachment of root hairs to soil particles (*after Molisch*).

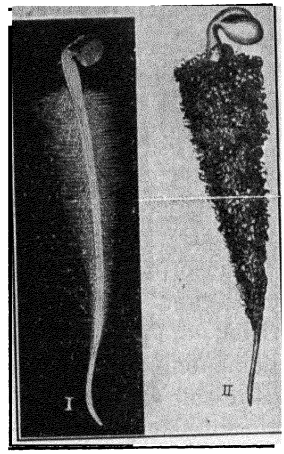


FIG. 75.—A radish seedling. I, showing root hairs; II, with a soil coating around the roots (*after Molisch*).

stration. A carefully polished marble plate is inserted horizontally or obliquely in the soil in which plants are grown, legumes being best suited for this purpose. When the roots have developed and some of them have come into close contact with

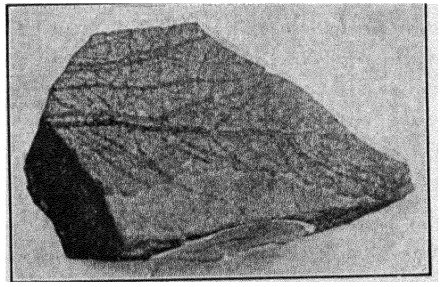


FIG. 76.—Marble plate showing traces left by roots (*after Nathansohn*).

the marble plate, it is taken out and washed. On the polished surface of the plate will now be seen distinctly small traces etched at the points of contact with the roots (Fig. 76).

It is possible to observe in the same way the dissolving effect of the roots upon natural rock phosphates. Sometimes, in water-culture solutions, nutrient elements are introduced in the form of solid substances, which are gradually used by the plants during their growth, as in Crone's solution, where phosphorus is supplied in the form of water-insoluble $Ca_3(PO_4)_2$ and $FePO_4$.

The mechanism of the dissolving action of roots is not understood as yet. An important function is performed by CO_2 excreted by the roots. It increases the solubility of many substances in water, especially the carbonates. Some investigators (Mazé, Schulov) have found that roots excrete malic acid as well as sugar and other organic substances. In the dissolving of insoluble substances, an important part is played by the physiologically acid salts, for instance, ammonium sulphate (see Art. 50). Roots of plants absorb more of their cations than anions, which causes an increase in the acidity of the medium. Prianishnikov has shown that the roots of cereals can dissolve solid rock phosphate only in the presence of nitrogen in the form of ammonium sulphate. If fertilized with nitrates, the roots are not able to dissolve insoluble phosphates.

An important factor in dissolving mineral substances in the soil is the activity of bacteria. Many of these soil organisms, for example, the bacteria, that cause the disintegration of cellulose among other products, excrete acids, chiefly butyric and lactic. Hence the solution of rock phosphate proceeds more easily and rapidly in humus soils rich in bacteria than in poor soils. In order to separate the activity of roots from those of bacteria, experiments must be carried out in sterile cultures. Unfortunately, there is not at present a technique that would allow the sterilization of soil without fundamentally changing all its properties; that is why it has not been possible to apply sterilization methods in soil-culture experiments.

The concentration of hydrogen ions in the soil likewise affects the availability of the insoluble substances. According to experiments first carried out by Engelgard, cereals absorb rock phosphate better from acid podsol soils than from slightly alkaline black soils, as the amount of calcium bicarbonate is one of the most important factors regulating the availability of the insoluble substances. It has been observed that an excess of calcium salts, which produces an alkaline reaction of the soil,

causes chlorosis in many plants, the iron salts having been changed to an insoluble form.

60. Methods of Determining the Nutritive Value of Soils. Methods of Pot and Field Experiments.—The complexity of relations accompanying the absorption of mineral nutrients from the soil does not permit one to determine exactly the degree of its fertility solely on the basis of chemical analysis. Upon complete analysis, there will usually be found even in the poorest soils such quantities of the necessary elements as will not only satisfy but be in excess of all the needs of a plant. An analysis does not give an answer to the question as to whether these elements are present in available or unavailable form. It gives an idea, however of the potential amount of mineral nutrients that can be made available to the plant through the processes of weathering. An analysis furnishes sufficient data only on nitrogen, as this constituent of the soil is comparatively easily and rapidly transformed into substances accessible to plants. In peat-bog soils, however, even nitrogen is so firmly bound that it is unavailable to the roots of plants, and chemical determination of nitrogen does not give a true picture of the soil's fertility.

Results of a complete analysis usually give an exaggerated idea of the quantity of available mineral substances that are present. On the other hand, analysis of soil extracts or of artificially prepared water solutions does not give a correct idea of the nutritive qualities of a soil. The value thus secured usually is too small, for the roots have a higher capacity of dissolving substances than pure water.

In order to learn more exactly the nutritional capacity of the soil, it is often extracted with 1 per cent hydrochloric acid or with various concentrations of citric acid or ammonium citrate. This method is especially useful for determining the amounts of available phosphates. Data are thus secured that more closely correspond to availability of nutrients from the soil, but nevertheless these are only approximations. This is particularly the case when it is considered that the various plants possess different abilities to absorb nutrients.

These defects in chemical methods of soil analysis have led to a search for other methods, in which the plant itself would serve as an indicator of soil fertility. The method of pot cultures is valuable in this respect. It has been worked out by investi-

gators in agricultural experiment stations—Hellriegel, Neubauer, and Wagner in Germany; Kossowitch and Prianishnikov in Russia. The principle of this method is the cultivation of plants on natural soils in special glass, clay, or metal pots holding from 5 to 100 kg. or more. The environmental conditions are kept under control as far as possible. The soil moisture is held constant by means of daily watering of the pots to a constant weight. The pots are filled with equal amounts of carefully mixed soil

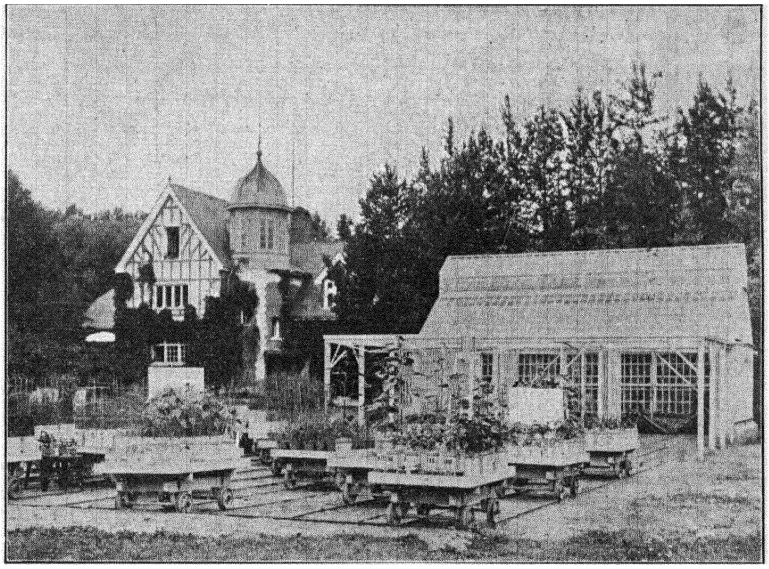


FIG. 77.—Greenhouse for pot experiments at the Institute of Applied Botany in Detskoje Selo, near Leningrad.

and are placed on rolling platforms, which may be pushed outdoors during good weather and back into a special greenhouse to avoid wind, dust, and rain (Fig. 77).

Soils are commonly tested in the following way. A certain number of containers, usually two to four, are fertilized with all the necessary ingredients in which a soil may be deficient, usually N, K, and P, since Ca, Mg, and S are present in sufficient amounts in almost every soil. An equal number of containers remain unfertilized. These two series serve as controls, the first showing the maximum yield obtainable under the conditions of the experiment, the second, the minimum yield. The rest of

the containers are fertilized with one of the elements mentioned. For instance, one series receives only nitrogen; another, potassium; and a third, phosphorus. The experiment is continued throughout the vegetative period, and at the end of it the appearance of plants in the different series is compared.

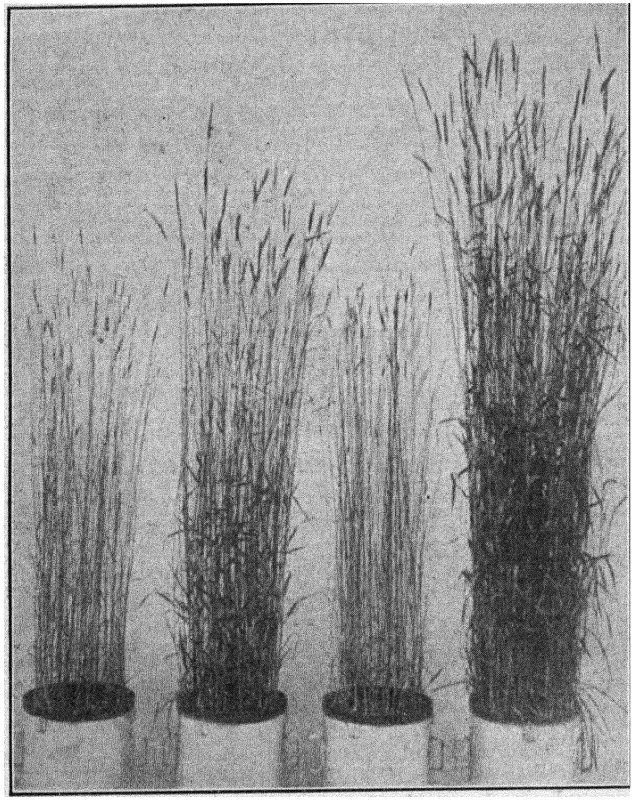


FIG. 78.—Effects of different fertilizers on wheat in pot cultures. On the extreme left without the fertilization; one next to it fertilized with nitrates; the third with potassium and phosphorus salts. The pot on the extreme right has received complete fertilization (*after Wagner*).

If the addition of one of the fertilizers, for instance, nitrogen, increases the yield close to its maximum, this shows that the soil is especially in need of nitrogen. If the same result is obtained by adding phosphates, the soil is deficient in phosphorus. If the addition of only one element is not sufficient to secure the highest yield, then the experiments are carried out with com-

binations of the various fertilizers, *e.g.*, K and P, K and N, P and N. From the combinations that give the best results, one can judge in which of the elements the soil is most deficient. By means of such experiments, Wagner succeeded in demonstrating very clearly the capacity of leguminous plants to thrive on the nitrogen of the atmosphere. He discovered that legumes do not respond to the application of nitrates (Figs. 78 and 79).



FIG. 79.—Influence of various fertilizers on peas in pot cultures. Pot on extreme left without fertilizers; one next to it fertilized with nitrates; the third with potassium and phosphorus salts. The pot on extreme right has received complete fertilization (*after Wagner*).

Though the pot-experiment method is very valuable in indicating which of the nutrient substances is present in the soil and which fertilizers must be added, it cannot furnish correct quantitative data; for in pots, the roots of plants are confined to a volume of soil considerably smaller than when grown under field conditions. This is the reason why the requirements for fertilizers of plants in pots are always higher than those of plants in the field. For exact determination of the amounts of fertilizer to be added, it is expedient, therefore, to use the field method.

An experimental field is usually divided into plots of a definite size, and to each is applied either one fertilizer or a combination of fertilizers. By determining separately the yield from each plot, it is easy to find what the fertilizer requirements are. This procedure of determining the needs of a soil is widely used by experiment stations and in experimental field work. Expressing it figuratively, Timiriazeff said that in applying this method we ask the plant directly in what a given soil is deficient for its welfare.

A still more rapid answer to the question of how much substance and what substances the plant will receive from a particular soil is given by Neubauer's method. The procedure of this method is as follows. A great number of seedlings (usually 100) are grown for 2 to 3 weeks in a flat glass tray on a small amount of soil (100 g.). The plants are then separated from the soil, dried, and carefully analyzed. It is supposed that the plants have had time to absorb all of the available mineral substances from the soil and that the quantity of the mineral elements, particularly phosphorus and potassium, shown by the analysis, after the amount present in the seeds has been deducted, corresponds to the amounts of these elements in the soil that are available to the plants. Neubauer's method is no doubt very ingenious, but evidently it cannot be applied to all soils. Moreover, the very small amounts of the substances that have to be determined call for very delicate and very exact methods of chemical analysis. Then, too, in making calculations from 100 gm. and applying them to acre plots, every error of the analysis is multiplied many thousand times. At present, this method is not very generally used.

Very convenient and rapid is the method of determining the nutritive value of soils by growing in them the mold *Aspergillus*. This method was suggested by Butkevitch (1909) and has been elaborated in detail by Nicolas. It is based on the fact that molds react just as conspicuously as higher plants to the presence of the indispensable mineral elements. The weight of the mycelium of these fungi serves as a qualitative index of the amount of elements, especially potassium and phosphorus, contained in the soil. Some sucrose and citric acid are added to the soil to satisfy the requirements of these organisms for organic substances.

Before the application of artificial fertilizers, it is necessary to learn which of the elements are needed for a certain soil. In contrast to the natural fertilizers, such as manure or the various organic products, by the term "artificial fertilizers" is understood the different mineral compounds obtained in part from the soil itself, *e.g.*, Chile saltpeter, rock phosphates, and Stassfurt potash, or secured as products or by-products of various industries. Ammonium sulphate, for instance, is obtained in the process of the purification of illuminating gas, and Thomas slag is formed during the smelting of ore containing phosphorus. Some artificial fertilizers are the products of direct synthesis, such as synthetic nitrates or ammonia. Natural fertilizers contain all the substances necessary for the plant, while artificial fertilizers are unbalanced, usually furnishing only one, two, or three of the needed elements. It is clear, therefore, that the application of a potash fertilizer to a soil infertile as a consequence of phosphorus deficiency, is a useless expense and will not lead to an increased yield. Naturally, the transition from a primitive type of agriculture, based principally on fertilization with manure, to more intensive forms, in which the application of artificial fertilizers becomes common, requires the development of a system of experiment stations, where the requirements of the local soils can be studied in connection with the needs of different crops, such experiments being beyond the capacity of individual farmers.

The application of artificial fertilizers is subject to the law of the minimum, first established by Liebig (1846) at the time of the introduction of artificial fertilizers into agricultural practice. According to this law, as worked out by Hellriegel, the yield is determined by the amount of that necessary element which is present in a minimum quantity in proportion to the demands of the plant. If the content of this element is gradually increased, the yield will be increased in proportion to the amount applied until another element begins to act as a limiting factor.

The law of the minimum has been applied not only to the influence of mineral elements on yield, but to all factors that determine the development of plants, such as light, water, and temperature. In this modified usage, this law has served as a foundation for Blackman's law of limiting factors, which was mentioned in Art. 47.

The law of the minimum established by Liebig has played an important role in the development of fertilizer practice and is one of the great discoveries of this scientist. But numerous investigations of many scientists later on established with certainty that the different fertilizers do not act independently of each other, as was stated by Liebig and his followers, but that complicated interrelations may be observed in their activity. The plant is not indifferent to the relative proportions in which it obtains the necessary elements. It is known, for instance, that an increase in the amount of nitrogen augments the requirements of the plant for phosphorus and potassium and that the requirements of plants for mineral elements are greatly influenced by climatic conditions, in particular by moisture conditions, etc.

61. Consumption of Mineral Elements by Different Plants. The Accumulation of Mineral Elements by Plants at Different Stages of Growth. Relation of Plants to the Content of Calcium Salts in the Soil.—The chemical composition of the ash of plants is variable, depending largely on the environmental conditions under which the plants are grown, their general properties, and their age. It has already been noted that the dryer the climate and the greater the amount of salts in the soil, the higher will be

THE COMPOSITION OF ASH OF SOME AGRICULTURAL PLANTS

Plants	K_2O	Na_2O	CaO	MgO	Fe_2O_3	P_2O_5	SO_2	SiO_2	Cl
1. Seeds:									
Wheat	30.2	0.6	3.5	13.2	0.6	47.9	...	0.7	
Corn	29.8	1.1	2.2	15.5	0.8	45.6	0.8	2.1	0.9
Clover	35.3	0.9	6.4	12.9	1.7	37.9	2.4	1.3	1.2
Flax	26.7	2.2	9.6	15.8	1.1	42.5	...	0.9	
Beans	41.5	1.1	5.0	7.1	0.5	38.9	3.4	0.6	1.8
2. Stems and leaves:									
Wheat	13.6	1.4	5.8	2.5	0.6	4.8	...	67.5	
Corn	27.2	0.8	5.7	11.4	0.8	9.1	...	40.2	
Clover	27.2	0.8	29.3	8.3	0.6	10.7	...	6.2	
Flax	34.1	4.4	24.8	15.0	3.7	6.2	...	6.7	
Buckwheat	46.6	2.2	18.4	3.6	...	11.2	...	5.5	
3. Tubers and roots:									
Potatoes	60.0	3.0	2.6	4 9	1.1	16.9	6.5	2.1	3.4
Sugar beets	53.1	8.9	6.1	7.9	1.1	12.2	4.2	2.3	4.8
Turnips	45.4	9.8	10.6	3.7	0.8	12.7	...	1.8	5.0

the ash content of a plant. One cannot conclude from this, however, that accumulation of ash in the plant is merely a mechanical process and simply the result of evaporation of water from the leaves, the salts dissolved in it being left in the plant. Plants growing even close together on the same soil always show differences in ash composition, thus indicating clearly the selective accumulation of some elements in preference to others. Likewise, in different parts of the same plant, the composition of the ash varies considerably.

The table on p. 264 contains data on the composition of ash of the vegetative organs and seeds of some important agricultural plants. As is customary, the percentages are calculated not on the basis of pure elements, but as their oxides; and the amount of each oxide is given as the percentage of the total weight of pure, carbon dioxide-free ash.

The table shows that seeds are rich in phosphorus and potassium, elements very necessary for the building of new organs and very often lacking in the soil. The stems and leaves of cereals often show a high silicon content, and those of flax and clover, a high calcium content. In the subterranean storage organs, large amounts of potassium are present. This seems to suggest an important role of this element in the storage of carbohydrates.

Because of the varying ash content and composition and the different capacity of plants to feed upon slightly soluble soil compounds, they naturally possess different requirements for soil nutrients and need, therefore, different fertilizers. Potatoes, for instance, often respond to potash, and cereals to phosphorus. Moreover, in agricultural practice, various amounts of ash elements are removed with the harvest, and therefore various crops exhaust the soil to a different degree. The average quantities of ash elements extracted from the soil by certain agricultural plants are given in the following table, the figures indicating the number of kilograms per hectare.

Plants	K_2O	CaO	MgO	P_2O_5	SO_3	SiO_2
Winter cereals	50	14	9	23	5	106
Spring cereals	50	18	10	20	6	86
Legumes	60	60	16	27	10	10
Potatoes	106	35	20	33	16	8
Beets	184	40	27	32	12	6

The table (p. 265) shows that highly cultivated crops, like potatoes and legumes, exhaust the soil more than do the cereals and thus increase the fertilizer requirements of the soil. Legumes, therefore, cannot be regarded as crops that only enrich the soil. Though increasing its nitrogen content, they exhaust the mineral reserve.

In farm practice, where almost all the mineral elements taken from the soil are returned to it in the form of manure and other refuse, soil exhaustion takes place slowly. But with the development of modern marketing, agricultural products are not used locally but are sold mostly in the city, and the mineral substances contained in the crop are lost forever. Therefore, the farmer must return to the soil the substances removed. This statement was clearly formulated for the first time by Liebig and termed by him "the law of return." He took this law literally, stating that without exception all the mineral elements removed from the soil must be returned, preferably in the form of artificial fertilizers, which he was the first to introduce into agricultural practice. In his first artificial fertilizer mixtures, he had all the ash elements, including Mg, Na, S, and Si.

It was soon found, however, that the law of return must not be taken literally and that it is quite unnecessary to return some of the elements, for inexhaustible reserves of them are contained in the gradually disintegrating rocks. That is why at the present time usually phosphorus, potash, and nitrogen are returned to the soil, without much attention being paid to the other elements.

Plants do not absorb the elements at the same rate during the different stages of growth. As a rule, most of the annuals absorb the greater part of the salts necessary for their development before the flowering stage. In some plants, as for instance corn, all the soil nutrients required for their further development are already accumulated in the stem at the time of flowering, when their further absorption ceases almost completely. There is also a difference in the rate of absorption of individual elements during the development of the plants.

Thus, for instance, according to Arendt's data, potassium and calcium are absorbed by oat plants chiefly during the first half of their vegetation, while the absorption of magnesium is very uniform throughout their growth period. Dividing the vege-

tative period of oats into five sections, the author observed in the first period 39 per cent of the total amount; in the second, 31 per cent; in the third, 21 per cent; in the fourth, 9 per cent; and in the fifth, 0. For calcium, the corresponding figures were 30, 28, 21, 20, and 1 per cent; for magnesium, 24, 18, 16, 26, and 16 per cent. Like magnesium, phosphoric acid and nitrogen are absorbed fairly uniformly throughout the vegetative period of oats.

The time of absorption of different elements varies rather widely for plants of different types. Thus, for instance, the curve of the absorption of ash elements for oats, being very high initially, falls rapidly; for peas, on the contrary, it rises continuously to the end of vegetative growth. Probably this is connected with the fact that oats complete their growth soon after heading; while in peas, flowering is prolonged, and the plant has a more or less continuous type of development.

Toward the end of the vegetative period of annuals, when synthetic activity has ceased and only processes of translocation of accumulated substances into the seeds take place, considerable losses of ash elements may frequently be observed. According to André and other authors, the loss of potassium may reach at the end of the vegetative period up to $\frac{1}{3}$ of the maximum amount accumulated; the loss of calcium, up to $\frac{1}{5}$; and that of magnesium, up to $\frac{1}{10}$. It is probable that a part of these losses may be attributed to the washing out of salts from dead leaves by rain, but certainly a considerable loss is chargeable to the backward translocation of substances into the root system. It is interesting to note that the greatest translocation is observed with potassium, which is present in the plant almost entirely in the ionic state, since it does not form any complex compound with organic substances. Most plants are capable of growing on different soils, for almost all contain sufficient nutrient substances for their development. Hence, climate permitting, it is possible to raise the same crop plants in widely differing soil zones. Still, not all soils can satisfy the requirements of different plants to the same degree, and the distribution of the wild flora can often serve as an excellent indicator of soil properties. A few characteristic plants may be considered as definite indicators of certain soil conditions, not only of its chemical composition but its physical character and water-holding capacity as well.

Plants are greatly influenced by the calcium content of the soil, especially by calcium carbonate. In their relationship to these salts, plants may be divided into two large groups: those that have a high requirement for lime, or calciphiles; and those that avoid limestone soils, or calciphobes. These two groups are linked by intermediate plants. They can be sharply separated only by considering their extreme representatives. These differences, however, are of a purely physiological character. They cannot be distinguished by any anatomical or morphological peculiarities. Not many of the higher plants are definitely calciphobes. Good examples of such plants are the lupines, the chestnut (*Castanea vesca*), sphagnum moss, and several other plants of sphagnum bogs.

As calcium represents one of the indispensable elements for the development of higher plants, it seems at first sight incomprehensible how there can be plants that do not grow on calcareous soils. Yet the ash of such lime-avoiding plants contains normal amounts of CaO. This becomes understandable, however, if it is remembered that an increased or decreased lime content results in chemical and physical changes of the soil. Soils that contain much lime have a neutral or slightly alkaline reaction advantageous to bacterial activity and the accumulation of humus, which is favorable to plant growth; while in acid soil, fungi develop, and an acid humus accumulates, which is injurious to many plants. The increase in calcium carbonate also improves the physical properties of soils, such as their porosity and their heat absorption. It is interesting to note that in water cultures all plants, calciphilous as well as calciphobous, require the addition of a sufficient amount of calcium ions for their normal development. With sufficient acidification of the solution, even the most calciphobous plants grow perfectly well in a high concentration of calcium salts. Conversely, upon alkalization of the soil solution even a small surplus of calcium is injurious. Thus the relation of plants to lime is closely connected with their relation to the concentration of hydrogen ions in the soil.

62. Effect on Plants of the Hydrogen-ion Concentration of the Soil.—Of all the cations affecting the development of plants, H^+ ions are the most active. One of the principal properties of acids and alkalies, as well as water, is the faculty of dissociating

into cations and anions. In water, as well as in any aqueous solution of acid or alkali, therefore, a certain number of free ions, H^+ and OH^- are present, and the so-called "neutral reaction" indicates not the complete absence of hydrogen ions but the equal concentration of hydrogen and hydroxyl ions. Water is then a perfectly neutral medium. Water dissociates into H^+ and OH^- ions according to the law of mass action.

$$\frac{H^+ \times OH^-}{H_2O} = K;$$

That is, the product of the concentrations of H^+ and OH^-, divided by the concentration of undissociated molecules H_2O, equals a constant K, called the "dissociation constant of water."

Since the concentration of undissociated water molecules H_2O, in water and in weak aqueous solutions, is much higher than the concentration of H^+ and OH^- ions, H_2O may be regarded as constant in water, as well as in aqueous solutions of acids and alkalies. Then the product of the concentrations of H^+ and OH^- will be equal to the constant $H^+ \times OH^- = Kw$. The determinations of different investigators have shown that at 22°C., the dissociation constant for water is $Kw = 10^{-14.14}$ gram molecules per liter. In a neutral solution, the concentrations of hydrogen and hydroxyl ions are equal; consequently, the concentration of $H^+ = 10^{-7.07}$. As H^+ and OH^- ions are present in both acid and alkaline solutions, but in different proportions, the product of their concentrations being a constant, not only the acidity but also the alkalinity, may be expressed by indicating the concentration of the H^+ ions. The active acidity C_{H^+} of a solution is usually indicated not in gram equivalents per liter or normality, but by the negative logarithm of the concentration of H^+, denoted by the sign pH, the hydrogen-ion exponent. Thus, the concentration of the H^+ ions at neutral reaction is denoted by pH = 7. As pH is a negative logarithm, the lower the designation of pH, the higher the concentration of the H^+ ions and the more acid is the reaction; and vice versa, the higher the pH is, the more alkaline is the reaction.

The development of plants is in general possible only within comparatively narrow limits of concentration of the H^+ ions.

The pH value of the soil may vary from 3 to 9. The first figure indicates a very acid soil; the second, a strongly alkaline

one that can only be attained by the accumulation of sodium salts. Calcium salts can increase the actual acidity of the soil only to pH 8.3. Chernozem[1] soils, rich in carbonates, have also an alkaline reaction; podsol[2] soils, from which all soluble bases are washed out, on the contrary, are acid. Soils of bogs are usually very acid.[3]

Recent investigations have shown that the hydrogen-ion concentration is one of the essential factors in determining plant distribution. Some plants grow best at one pH concentration, others at another. Very often it is possible to judge more or less exactly of the active acidity of the soil from the specific association of a meadow vegetation. For instance, the Danish investigator Olsen (1923) has proved that the predominance of *Deschampsia flexuosa* among the meadow grasses indicates a pH of 3.5 to 3.9; *Carex goudenougii*, a pH of 4.5 to 4.9; *Deschampsia caespitosa*, a pH of 5.5 to 6.4; and *Tussilago farfara*, a pH of 7.5 to 7.9. These figures have been confirmed by artificial cultures of these plants in nutrient solutions. It has been shown that each plant has its optimum, maximum, and minimum concentration of pH for growth (Fig. 80).

The numerous investigations of the last few years, especially the work of Arrhenius, have helped to elucidate the response of plants to the reaction of the soil. It has been ascertained that different species show different requirements with regard to the optimum concentration of H^+ ion and are moreover distinguished

[1] Chernozem (black earth): A very productive soil developed under subhumid or semiarid climatic conditions on open grassland. The soil is characterized by a very dark surface horizon that varies in depth from 1 to 4 ft. and that possesses a well-developed granular structure. The soil does not contain soluble toxic salts, but both the organic and mineral colloids are saturated with calcium and magnesium. The normal reaction is neutral and the typical topography smooth. The largest continuous area of chernozem lies in the southern half of Western Europe (Russian steppe) and extends eastward into Siberia.

[2] Podsol (ashlike soil): A soil developed under forests of moderately humid regions. It is characterized by an ashy gray or nearly white horizon just below the leaf litter. The horizon varies in thickness from several inches to more than 2 ft. Below the ashy-gray, or podsolized, horizon lies a very heavy, compact, dark-brown or reddish-brown horizon of varying thickness, usually 1 to 2 ft. The podsolized horizon is typically acid in reaction. The topography varies from undulating to rough. Typical podsols are to be found in the forested portion of Northern United States.

[3] Bogs in Northwestern United States are frequently alkaline.

by a different degree of resistance to changes in this connection. While oats, rye, turnips, and potatoes yield the highest returns with a pH of about 5 to 6, wheat, barley, beetroot, and alfalfa thrive better with neutral or slightly alkaline reaction. Not only different species, but even different races of the same plant, show different requirements with regard to pH.

The physiological influence of the actual acidity in soils is still very poorly explained, and great divergence exists in the views that are held among different investigators. It has been established that the pH exerts a great effect on the penetration

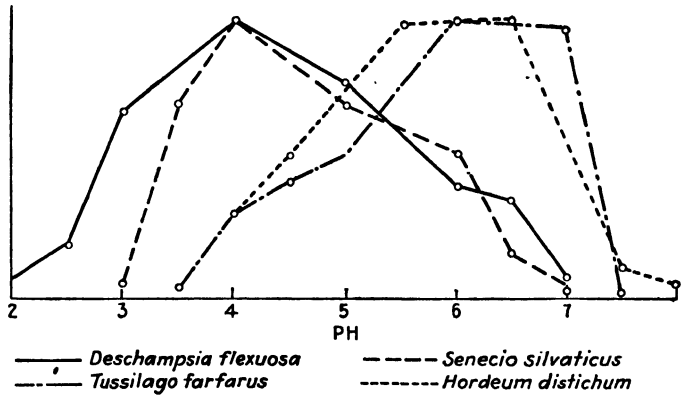


FIG. 80.—Growth of several plants in solutions of different hydrogen-ion concentration (*after Olsen*).

of salts into the cell; the increase of acidity promotes the absorption of anions, while the increase of alkalinity, on the contrary, promotes the absorption of cations. On the other hand, there is a hypothesis that at optimum pH for a given plant, the permeability of the protoplasm to salts is the least; and that with the shifting of pH to the one or to the other side of this optimum, the permeability may increase too much, resulting in injury to the plant.

The soil, which is the natural medium for the development of plants, represents a much more complex substratum than the solutions used in water and sand cultures. According to many authors, the influence of the pH of the soil may be directly upon the plant or may act indirectly by affecting the solubility of the nutrient or toxic soil substances. Thus, it is known that acid soils or physiologically acid fertilizers applied to the soils promote

the solubility and absorption of phosphates. The solubility of the iron and aluminum salts equally depends on the pH of the soil. The investigations of Uspensky with algae have shown that the more iron the organism requires, the more the pH optimum for its development shifts to the acid side, since the solubility of iron increases with acidity. Conversely, algae that are susceptible to an excess of iron, *e.g.*, *Cladophora*, are injured in consequence of a high iron content in water the reaction of which is lower than pH = 7. Similar conditions may hold for higher plants. The chlorosis of some plants on alkaline soils rich in calcium carbonate may be explained not so much by the injurious influence of the reaction as such, as by the lack of iron that is transformed in an alkaline medium in the presence of calcium and phosphates into an insoluble form. Chlorosis is observed in the soil less frequently than in water cultures, since the soil is usually rich in organic substances that produce complex compounds with iron. These compounds are retained in the solution even with an alkaline reaction.

On the other hand, the pH of the soil may influence the plant by acting on the solubility of the poisonous Al^{+++} ion. Generally, in neutral soils aluminum is quite insoluble, but in acid and in alkaline soils, the solubility of aluminum sharply increases. Plants may suffer greatly in acid and in strongly alkaline soils, not only from the H^+ or OH^- ions, but also from the toxicity of aluminum. At the present time, these questions are being investigated in detail by physiologists as well as by soil scientists, but they are still far from being solved.

Besides the actual acidity determined either electrometrically or by indicators as described in physical chemistry, the potential or titratable acidity, determined by the usual methods of titration, may have an influence on plants. Solutions with the same actual acidity may show different amounts of titratable acidity, depending on the degree of dissociation of the acids in the solution and their buffer action. "Buffer action" is the property of the solution to minimize changes of the H^+ concentration on dilution or on the addition of H^+ or OH^- ions. Buffer action depends on the presence of mixtures of weak acids in the solution, or of weak bases, with their salts. Well-buffered solutions may be mixtures of carbonates of alkalies, or alkaline earth metals with carbon dioxide, or mixtures of phosphates of different basicity

in which there are potential reserves of anions and cations able to ionize and neutralize the H^+ and OH^- ions. The soil always contains, to a greater or less degree, the above-mentioned combinations. For this reason, a soil solution is much more stable in regard to pH than aqueous solutions. The soils showing the greatest buffer action are those rich in organic substances, *e.g.*, peaty or muck soils. Soils showing the least buffer action are sandy soils, gray leached soils, and podsols.

Besides the actual acidity, soils also possess potential acidities apparent only when the soil is fertilized by the application of mineral fertilizers. This form of acidity is explained by the adsorption of the added salts with the liberation of free acids or by the replacement of the base of the salt by aluminum. The newly formed salt is hydrolyzed in turn into the slightly dissociated aluminum hydroxide and the strongly dissociated free acid, which determines the concentration of hydrogen ions. The significance of these types of acidity for plants has not been fully studied.

63. Physiological Basis of Fertilization.—It is customary to regard fertilizers as nutritive substances, the addition of which to the soil directly contributes to an increase of the yield. As a matter of fact, a much more complicated phenomenon is met with here. It has already been emphasized that only a part of the substances absorbed by the roots, chiefly nitrogenous substances as well as anions of phosphoric and sulphuric acid, should be regarded as substances participating in the composition of the most important constituents of the protoplasm, the protein substances. Only these elements should be compared in importance with the source of carbon, the carbon dioxide of the air. The main role of their action is the regulation of the vital processes, and their influence on the accumulation of organic substance by the plant is mostly indirect.

This is obvious from the fact that the introduction of fertilizers into the soil leads not only to an increase in the plant of the total amount of ash and nitrogen, but far more to an increase of the total quantity of organic substance, most of which consists of carbohydrates, which of themselves do not contain either mineral elements or nitrogen. That is why it is necessary to search for an explanation of the action of ash elements, especially of cations, upon the yield through their influence upon the vital processes

contributing to the accumulation of organic substances in the plant.

As has already been seen, the basic process of the creation of organic from inorganic substances is photosynthesis. It might be assumed therefore that the introduction of mineral elements necessary for the nutrition of the plant, especially of potassium, which is supposed to participate in some way in the transformations of carbohydrates, would considerably increase photosynthesis. However, exact experiments carried out by Gassner (1933) have shown that with decreasing doses of potassium, the intensity of photosynthesis does not decrease but on the contrary increases, and only a strong deficiency in potassium suppresses the photosynthetic activity. Of all the elements absorbed by the root system, only nitrogen induces a perceptible increase in photosynthesis.

The interrelations between the mineral elements and the accumulation of organic substances, therefore, are very complicated. Mineral substances participate actively in the formation of new masses of living protoplasm, especially substances such as potassium, magnesium, and phosphorus, which are always present in more or less considerable quantities in meristematic tissues. They contribute to the utilization of the products of assimilation in the structure of new cells, thus leading to an increased growth intensity and to the creation of new assimilating leaf surfaces especially in the first periods of the development of plants. The same effect is produced by nitrogen; it has been definitely established that deficiency in nitrogen leads to a repletion of the leaves with unutilized carbohydrates. An increase of the photosynthesizing cells leads to a considerable increase in the accumulation of organic substance of the plant as a whole and thus to an increase of the yield. The action of mineral substances may thus be compared with the action of hormones, which control and increase growth.

The accumulation of mineral substances in the plant is most intimately connected with the accumulation of organic substances; if, on the one hand, application of fertilizers increases the yield, the absorption of mineral elements on the other hand may be accelerated by increasing the rate of the accumulation of organic substances. Such a phenomenon has been observed by Jegorov. The removal of flowers, which caused profuse

growth of the vegetative organs and a general increase of the plant mass, led to an increase in the absorption of mineral substances from the soil.

This close connection between the accumulation of organic and mineral substances explains why in arid conditions black soils do not react to the application of fertilizers, while the same soils and under the same climatic conditions, but with the application of irrigation or in pot cultures, show a clear dependence of the magnitude of the yield on the amount of fertilizers applied. With a deficiency of water supply, the yield on chernozem soils depends upon the amount of available water, since the amount of mineral and nitrogen substances present in chernozem soils is more than sufficient to produce the maximum yield possible with the limited water supply. But as soon as the water supply is increased, as for instance by irrigation or the regulation of watering in pot cultures, growth is accelerated, the reserves of nutrient elements in the soil are no longer sufficient, and as a consequence the plants are affected by the application of fertilizers.

Since the mineral elements represent chiefly regulators of the vital processes, they are of great importance to the plant whose growth is dependent upon the supply of these elements. In its first stages of development, the plant utilizes chiefly the reserves of mineral salts stored in the seeds. During this period of its life, the growth of the plant is relatively slow, and therefore its requirements are not so great; but they increase rapidly up to the time of the most vigorous growth, which coincides with the period of blooming and the setting of fruit. The time of accumulation of the chief mineral elements, at least of potassium and phosphoric acid, corresponds in general to the time of accumulation of organic substance. The cessation of increase of organic substance during the ripening of the seed is accompanied as has already been noted by a backward flow of a part of the mineral elements from the plant into the soil.

The usual methods of application of fertilizers by plowing them into the soil before the sowing of seed does not fulfill the requirements of the plant. By this practice, the plant obtains the maximum quantity of fertilizer elements in the first stages of its development when it least requires them; while at the time when its requirements for nutrients increase, the amount of nutrients

has diminished. Furthermore, the main mass of roots descends to the deeper soil horizons, where the fertilizers do not penetrate. The phosphates, especially, remain concentrated in the upper soil layers. Based on these facts, attempts are being made at present to modify the customary practice of fertilization in such a way as to make the fertilizers available to the plants at later stages of growth. For this purpose, the fertilizers are applied either between the crop rows, or else they are introduced deeper into the soil so that the roots of plants reach them at a later stage. In some cases, it is desirable to apply phosphates in holes that have been driven around trees, so as to get deeper penetration. Where artificial irrigation is applied, the fertilizers are sometimes dissolved in the irrigation water. A more detailed examination of the different practices of fertilization belongs not to physiology but to soil science.

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CHAPTER VIII

WATER RELATIONS OF PLANTS

64. General Concept of the Water Relations of Plants. The Amount of Water Used by Plants.—As has already been seen in Chap. I, the normal functioning of the plant cell requires its saturation with water. In submerged plants, as for instance in algae, which perhaps are the most ancient chlorophyll-bearing plants on the earth, saturation with water is a natural result of the ecological conditions of their surrounding medium. But in plants inhabiting dry land, the necessity of maintaining the protoplasm of the cells in a sufficiently saturated state represents the most important limitations to their growth. It determines their structure and all their life activity. To maintain satisfactory photosynthetic activity, the chlorophyll-bearing cells of land plants must be in continuous contact with the surrounding atmosphere, which provides them with the indispensable carbon dioxide. However, such exposure results in evaporation of water from the cell into the surrounding air. Sunlight, which furnishes the plant with the energy necessary for photosynthesis, on being absorbed by the chlorophyll is taken up also by other cell structures and raises the temperature of the leaf, accelerating the rate of evaporation. There must be a continuous stream of water passing toward the chlorophyll-bearing cells to compensate for this loss. The seemingly stationary condition of saturation is maintained in the plant by the coordination of absorption and evaporation of water, thus creating water relations that are specifically different for each plant. This has been called by L. A. Ivanov the "water exchange" of the plant.

Very few plants, and those mostly of a very simple structure, such as mosses and lichens, can endure a prolonged interruption of the water supply. Mosses and lichens can survive long periods of desiccation in an air-dry condition. Among the higher plants, only a few representatives of the desert flora, such as the sand sedge, endure such desiccation. For most of the higher

plants, desiccation is lethal; their water expenditure must more or less be balanced by an equal intake, and their water content shows only small fluctuations. The "water balance" is the relation between the intake and expenditure of water in plants. One of the most important conditions for the normal functioning of higher land plants is the establishment of this water balance without a permanent and significant deficit. This requires a well-developed root system that will absorb water from the soil with sufficient rapidity and a well-developed conductive system that transports the water without impediment to the main evaporating organs, the leaves. The plant must be provided also with a system of protective tissues that on occasion can inhibit the loss of water. The structure of all of these systems of absorptive, conductive, and protective tissues is described in detail in textbooks on plant anatomy, and it is assumed that the student is acquainted with them.

The amount of water that passes through the plant is immense. Experiments have shown that during 1 hr. of a hot summer day more water passes through a leaf of our common plants than the leaf itself contains at any particular time. During the whole vegetative period, a sunflower or corn plant evaporates as much as 440 lb., or a barrel, of water (Fig. 81). Of the total amount of this water, the plant assimilates but an insignificant fraction. This becomes evident upon comparing the above figures with the amount of water entering into chemical reactions during the same period in the synthesis of carbohydrates. In the environment of Central Europe, for every kilogram of water absorbed only 3 to 4 g. of dry matter are formed. If it is assumed that the dry substance consists of carbohydrates and that half of this weight is made up by carbon, the other half consisting of the elements of water, then it will be found that of the 1,000 parts of water that pass through the plant, only 1.5 to 2 parts are used in the process of nutrition. The remaining 998 or 998.5 parts pass through the plant in order to compensate for evaporation and to maintain a sufficient degree of saturation in the tissues of the plant.

Such lavish expenditure naturally requires a no less rapid absorption of water. This task is performed chiefly by the roots. The real dimensions of the root system are much greater than most people assume on the basis of such meager evidence as is

revealed when a plant is dug up in the usual way. The greater part of the most active fine rootlets are usually torn off in digging. Rotmistroff, in Russia, and Weaver, in America, were the first to give a correct idea of the real extent of the root system, when all of its small branches remain intact. These authors have shown that even in cereals, like oats and wheat, the root system

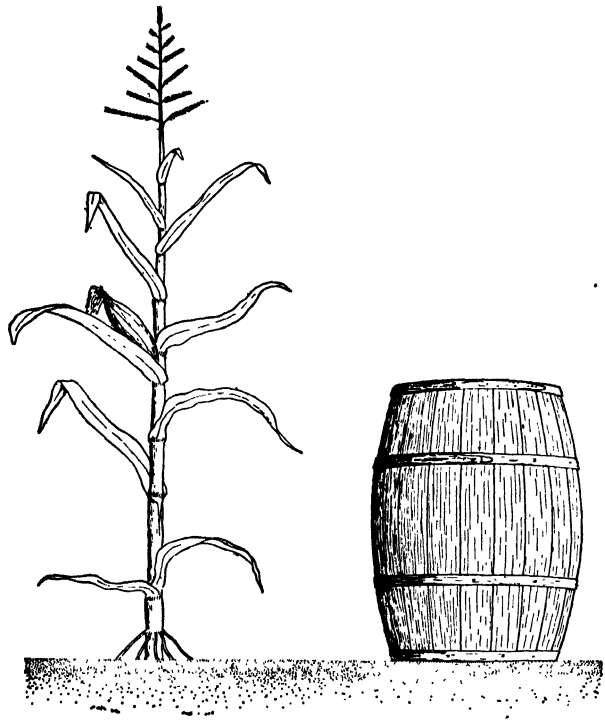


FIG. 81.—Amount of water evaporated during the summer by one maize plant (*after Sinnott*).

is not confined to the tilled layer. It penetrates into the soil to a depth of 1.5 to 2 m. and spreads extensively in all directions (Fig. 82). The roots of alfalfa and other plants of dry habitats penetrate considerably deeper. Even for small plants, the total length of the roots with all their branches measures several hundred meters. In larger plants, this length has to be measured by kilometers. The absorbing surface of the roots is increased several times by the development of root hairs. Such an

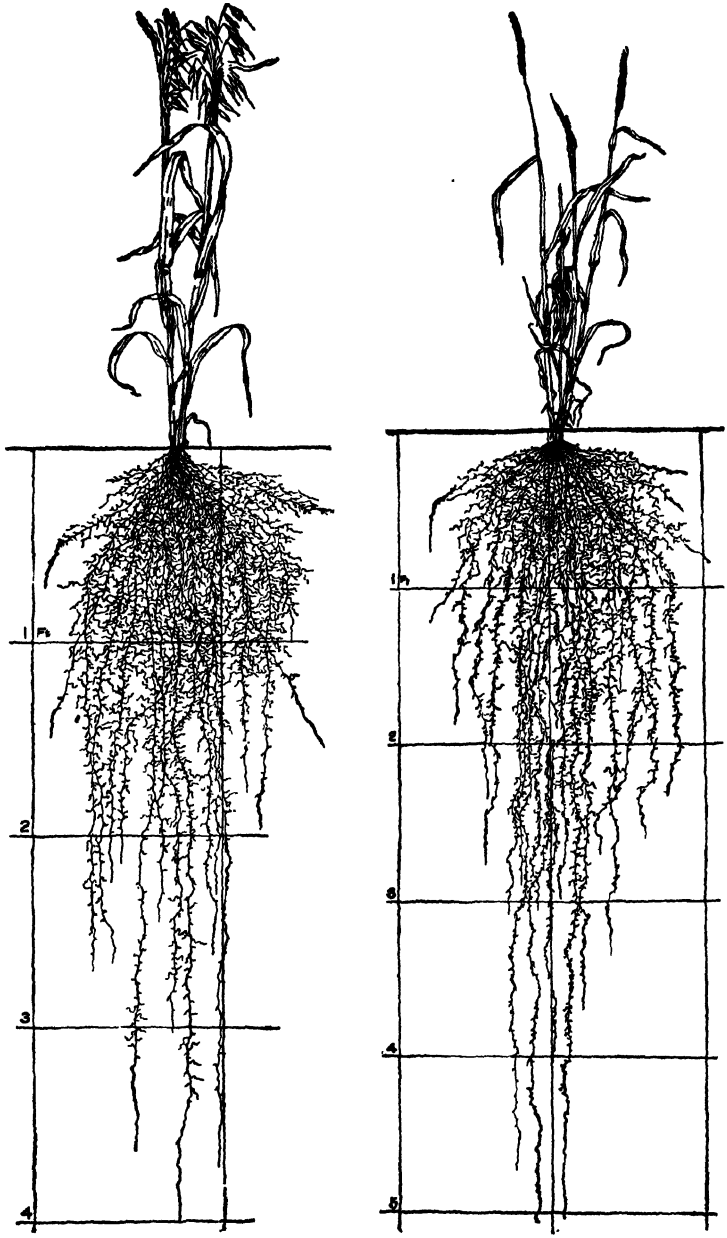


FIG. 82.—Oats and wheat in the flowering stage, with a root system carefully removed from the soil. The depth is shown in feet (*according to Weaver*).

immense system enables the plant to utilize perfectly even a scantily distributed soil moisture and equally meagerly distributed mineral substances.

The amount of water consumed by a plant during its life is not a constant magnitude but varies to a considerable degree according to the climatic conditions. In a hot dry climate, plants consume about two to three times more water than in humid climates. Similarly, under these conditions, they develop a relatively larger root system and a smaller evaporating leaf surface. Plants consume water least of all in humid shady forests, and it is just under these conditions that the larger and thinner leaves are found. Conversely, the root system of shade plants develops weakly, as does also the conductive system. That is why transpiration is sharply increased when the trees are cut down; shade plants desiccate and give way to light plants, which are better adapted to these new conditions.

Water expenditure and absorption are most intimately connected with each other and represent two phases of the same process, but here for convenience they will first be examined separately.

65. The Absorbing and Pumping Activity of the Root System. Root Pressure, Guttation, and Bleeding of Plants.—Land plants of simple structure, for example, the mosses and lichens, absorb water through their whole surface. In higher plants, special organs, the fine tips of roots or rootlets, serve this purpose. The cells of this part of the roots have thin walls made up of cellulose and callose. In the majority of cases, the cells have special outgrowths, the root hairs, which greatly increase the absorbing surface of the plant. Root hairs penetrate between the soil particles and absorb the water contained in them.

If the plant did not lose water continually by transpiration, the root cells would soon be saturated with water, and absorption would cease. The loss of water by the plant must, therefore, be regarded as the principal process inducing an unsaturated condition in the plant and causing a considerable suction tension. The plant as a whole presents, as it were, a self-regulating osmotic apparatus. The suction tension originating in the leaves is finally transmitted through a series of mechanisms to the root system, where it creates a water deficit that is necessary for the initiation of absorption.

Arising in the plant as a result of the physical process of evaporation, this suction tension is transmitted down the stem as a tension on the continuous water column in the conducting vessels and to the cells of the root. However, the latter possesses also a purely physiological means of forcing water upward through the conductive system. This is the phenomenon called "root pressure," which drives the water through the cells of the cortex and up into the vessels of the xylem. The path of the water ascending in the root may be represented by the adjacent diagram (Fig. 83). Into cell *A*, provided with a root hair, the water is absorbed from the soil and moved on to the cells *B*, *C*, etc., until it reaches cell *L*, adjoining vessel *M*. Here it is driven into the vessel and ascends the stem.

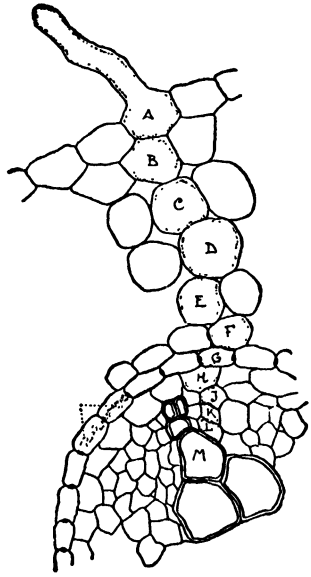


FIG. 83.—Path of water from the root hair *A* to the vessels of the root *M* (*after Priestley*).

To observe root pressure, a plant is cut above the soil. A rubber tube, ending in a glass tube is fastened to the cut stump.

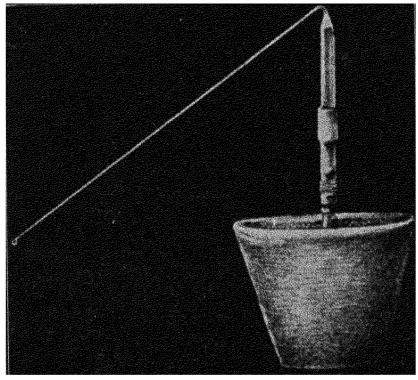


FIG. 84.—Exudation of sap from a cut as a result of root pressure (*after Molisch*).

The water excreted through the cut surface accumulates and rises in the glass tube. If the latter is bent down and a vessel

placed under it, the water exuding from the root system may be collected and analyzed (Fig. 84). This phenomenon has been called the "bleeding" of plants. The force that drives the water into the vessels, the root pressure, is produced by live cells in an active state that expend energy in the process. To measure this force, a mercury manometer is connected to the cut stump of the plant (Fig. 85).

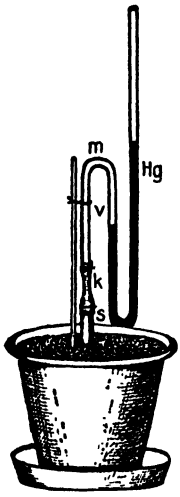


FIG. 85.—Measuring root pressure by means of a mercury manometer (*after Molisch*).

The phenomenon of bleeding is exhibited by various plants in different degrees. In some, like *Fuchsia*, nettle, and sunflower, it is readily detected. In others, for instance, the conifers, it is almost imperceptible. Bleeding shows also seasonal variations. It is most profuse in the spring, especially in woody plants, when in some of them, like the birch, maple, and grape, the sap flows abundantly from the cut stems and branches.

Root pressure may be observed likewise in uninjured plants. Seedlings of cereals, when placed in a moist atmosphere, accumulate water drops on the tips of their leaves. From time to time, these drops run down and are replaced by new ones. Similar drops may be observed at the margins of leaves of *Nasturtium* (Fig. 86), *Fuchsia*, or *Alchemilla*, and on the leaves of the potato and buckwheat. This phenomenon is known as "guttation." It is produced by the same root pressure that drives the water through the vessel of the leaf, just as through a glass tube. Especially strong guttation takes place in *Arum*, *Colocasia*, and *Philodendron*, plants commonly cultivated indoors. Under favorable conditions, such as high temperature and high air and soil moisture, the tips of their leaves may drip continually.

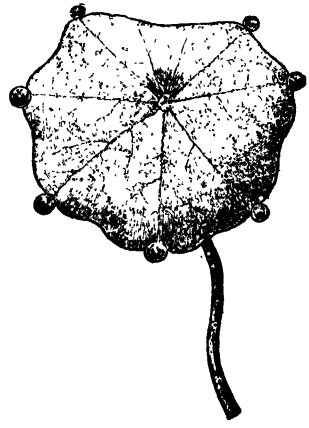


FIG. 86.—Guttation of a nasturtium leaf (*after Noll*).

The amount of water excreted under the influence of root pressure is generally insignificant. It does not exceed a few

cubic centimeters per day. The pressure developed in this process is also small. In herbaceous plants, it usually constitutes but a fraction of an atmosphere. Only in some trees and in the grapevine has a pressure above 1 to 2 atmospheres been measured. It must be remembered, however, that such experiments are usually conducted under conditions where the root system is separated from the aerial parts of the plant and, therefore, may soon weaken. As the water in the manometer accumulates but slowly, the recorded pressure is considerably lower than that which actually exists. The action of the root cells in producing bleeding and guttation is dependent upon the protoplasts being in an active condition, and in particular upon their osmotic properties. A simple experiment may serve as an illustration. A small pot with strongly guttating wheat seedlings is placed under a bell jar filled with vapors of ether or chloroform—or watering the soil with a solution of some poisonous substance may serve the same purpose. After a time, the root cells will be killed, and guttation will cease. The same results may be obtained by killing the roots with heat or by depriving them of oxygen. This result is easily understood, since it has been seen that all osmotic properties of the cell are closely dependent upon the normal structure and function of the protoplasmic membrane. With the death of the cell, this structure undergoes profound changes. The cell loses its semipermeability, along with its turgidity and suction tension. The dependence of root pressure on the life activity of the plant, however, has been found to be very complex. Not only a normal unaltered condition of the physicochemical properties of the protoplasmic membrane is required, but likewise an uninterrupted supply of nutrient substances. If this supply is checked, as when the aerial organs are cut, bleeding rapidly decreases or ceases.

To understand the cause of this phenomenon, the mechanism of the root pressure must be examined. It has been seen that the water enters the root under the force of suction tension and having passed through a series of parenchyma cells is driven with considerable force into the vessels. From the osmotic systems of the cell, however, no conclusion can be drawn as to the reasons for the possible expulsion of water from the cell. Although Dutrochet, who was the first to investigate osmotic phenomena in plants, compared the rise of the sap in the vessels of roots and stems to the rise of the liquid in the tube of an osmometer, the similarity is not very great. The tube of the

osmometer is the direct continuation of its cavity. The rise of the liquid, therefore, is simply the result of the general increase in volume of the solution filling the osmometer. In the root, on the contrary, the cavities of the vessels are separated from the adjoining cells through which the water is driven. Besides, it is not the cell sap but a considerably more dilute solution that ascends through the vessels. In order to visualize root pressure, it must be accepted that at least some root cells are able simultaneously to imbibe water and to expel it into the vessels. Therefore, the concept of the osmotic mechanism of the cell already discussed must be somewhat modified in order to understand this flow of water in one direction.

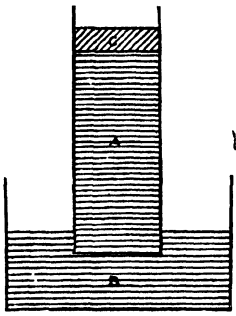


FIG. 87.—Diagram of water current passing through the cell in one direction (*according to Priestley*).

For this purpose, several modified mechanisms have been suggested. The idea that has recently gained wide recognition is the one chiefly put forward by Priestley, who considers that the water-absorbing cell, being saturated, develops no suction tension by itself. This is in perfect harmony with the experimental data of Ursprung, who has found that the suction tension of root hairs, when immersed in water, is zero. But the solution that is present in the cavities of the dead tracheal elements as a result of excretion by the living cells adjoining the vessels is not subjected to the pressure within the live cells. At this point, the solution therefore possesses a suction tension equal to the whole magnitude of its osmotic pressure. It must absorb water from the adjoining saturated cells, which begin to show a decrease in saturation and which because of suction tension absorb water from the surrounding solution. This shows that it is not the living cells that draw water from the soil, but rather the solution present in the vessels. The whole aggregate of living cells only filter the fluid and remain otherwise passive. The mechanism of absorption may be represented by the foregoing simple diagram (Fig. 87). One end of the cell *A* is immersed in a vessel of water *B*. On the other end is poured a solution *C*, of a certain concentration. Since cell *A* is saturated with water, solution *C* will draw water through it, quite independently of the magnitude of the osmotic pressure in cell *A*.

Priestley's scheme naturally requires that solution *C* come in contact with vessel *B* only through cell *A*. There should be no diffusion of the solution through the cell wall. But the walls of cells are commonly made up of cellulose, which is readily permeable to diffusing substances. Priestley, therefore, supposes that the role of an impermeable septum is played by the endoderm, the Casparian strip, whose corky radial walls seem to be formed for this function.

This scheme explains quite cleverly the mechanism of root pressure, as well as the role of the endoderm in certain parts of the root, the function of which before this was a puzzle. It also allows the measurement of the suction tension of roots by means of determining the concentration of the solution exuded from the cut stump of bleeding plants. According to Priestley's idea, the concentration of this solution controls suction tension. The determinations by Sabinin have shown that the suction tension of the roots of our common crop plants approximates 1.5 to 2 atmospheres.

66. Absorption of Water from the Soil. Water-holding Forces of the Soil. Available and Unavailable Water.—The absorption of water from the soil presents a much more complicated and difficult problem than the simple imbibition of water by roots immersed in a glass container. A number of forces exist in the soil that counteract the suction tension of the roots. The sum total of all the forces that retain water in the soil may be called the "water-holding capacity" of the soil.

It is not pure water but a solution of a certain concentration that is found in the soil and, therefore, of itself it exhibits a force that tends to hold water. If for convenience Priestley's mechanism is adopted, then it can be seen readily that with an equal concentration of the solution in the soil and in the vessels of the plant, all absorption must cease. But here, likewise, a special regulating mechanism exists in the plant, which indicates that the higher the concentration of the soil solution, the greater also is the concentration of the solution in the vessels of the plant. This mechanism, however, works only within certain limits. The majority of plants are unable to grow in strongly saline soils, where the concentration of the soil solution is very high. In such places a special flora develops, the so-called "halophytes." These plants have the faculty of accumulating an enormous

amount of salts and develop a very high osmotic pressure, up to 100 atmospheres or more.

Besides this osmotic resistance to absorption, another type of resistance due to adsorption is found in the soil. The soil consists chiefly of solid particles of various sizes together with more or less decayed organic matter of colloidal nature. Inorganic colloids also are present. The soil water is distributed in various degrees through these phases of the soil. Some of the water fills the larger spaces in the soil and remains in a rather mobile condition. This is called "gravitational water." It moves under the force of gravity, descending into the soil after a rainfall. In the smaller soil spaces, water is retained by the force of surface tension. This is called "capillary water," and it is held against the force of gravity. The force by which capillary water is retained in the soil is small, usually a fraction of an atmosphere, depending on the diameter of the space, or capillary. This water, therefore, is absorbed without difficulty by the root hairs, in general by the surface cells of the absorbing zone of the root. Gravitational water, of course, is taken up still more readily.

Water directly surrounding the soil particles is retained by the force of molecular attraction, or adsorption. This force is of a considerable magnitude. Moreover, it increases rapidly as the water film surrounding the soil particle grows thinner. Such a film of water is available to the plant only with difficulty. Finally, air-dry soil contains from 0.5 per cent in coarse sand, to 14 per cent in heavy clay, of so-called "hygroscopic" water. Hygroscopic water is retained by the soil particles with a force reaching as high as 1,000 atmospheres and is unavailable to the plant when the force with which it is held exceeds the suction tension of the cell.

The colloidal substances of the soil have the property of swelling in water. They have a considerable water-holding capacity. The more of these colloidal substances there are present in the soil, the more water is bound to them. The amount of this so-called "imbibitional" water is especially great in peat soils, which consist almost entirely of partly decayed plant residues.

In the soil, the root hair has to compete for water with the surrounding soil particles. The smaller these particles are and

the more colloidal substances there are present in the soil, the greater is the amount of water retained and unavailable to the root cells. This competition for water by the plants has been pictured by Sachs in the form of a diagram, showing the absorbing root hair among the water-retaining soil particles (Fig. 88).

To determine the amount of water in the soil that is unavailable to the plant, the following procedure may be used. When the plant under investigation has developed sufficiently, watering of the soil is stopped, and the pot is kept in a shaded place until the plant begins to wilt. Wilting indicates that the water supply

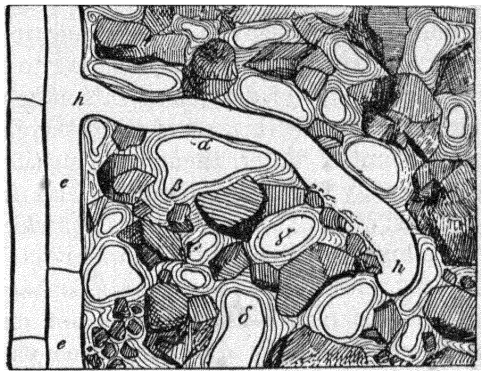


FIG. 88.—A root hair in the soil, absorbing water held by soil particles.

from the soil has ceased. At the moment of permanent wilting, the amount of water that still remains in the soil may be measured by taking a sample of the soil and drying it at 100°C. This is the water reserve unavailable to the plant, called "unavailable moisture."

The most detailed investigation of the amount of unavailable water in different soils was carried out by Briggs and Shantz. They investigated numerous samples of various soils and established a simple relationship between the amount of unavailable water, which they called the "wilting coefficient" of the soil, and the other water-holding properties of the soil, *e.g.*, the hygroscopic coefficient and the total water-holding capacity, as well as its mechanical composition. From their data, these authors proposed the following formulas for the calculation of the wilting coefficient q, or the unavailable moisture reserve of the soil, in percentages of its dry weight:

$$q = \frac{\text{percentage of hygroscopic water}}{0.68}$$
$$= \frac{\text{percentage of water at full saturation} - 21}{2.9}.$$

According to the data of mechanical analysis, the unavailable reserve may be calculated as follows:

q = percentage of sand $\times$ 0.001 + percentage of silt $\times$ 0.12 + percentage of clay $\times$ 0.57.

Thus, different soils show a very different wilting coefficient. For coarse sand, this coefficient is 1 per cent; for fine sand, 2 to 3; for loam, 5 to 10; for heavy clay soil, 14 to 16 per cent. Regarding the adaptability of different plants, it has been found that with sufficiently slow wilting, all of them leave in the soil a perfectly equal amount of water, though the suction tension of their root systems may be very different. This fact may be explained as follows: When the capillary water, which is readily available to all plants, is exhausted, the water-retaining forces of the soil rapidly increase to several atmospheres. As the suction tension of the plants usually does not exceed a few atmospheres, differences in this regard between various plants play almost no role. Moreover, the water loses its ability to move through the soil; hence the soil particles that are not in direct contact with the root hairs no longer give up their moisture to them, however great the suction tension of root hairs may be.

The data of Briggs and Shantz are in perfect agreement with those of Bogdanov, obtained at a much earlier date by studying the relation of germinating seeds to soil water. Bogdanov found that, independent of their nature and properties, the seeds of various plants can germinate only when the water content of the soil is no less than twice its hygroscopic capacity. His method of calculating the unavailable water has been made use of in agronomic practice. In calculating the amount of soil water available to the plant, usually twice the amount of hygroscopic water is subtracted from the total amount present.

67. Influence of Environmental Conditions on the Absorbing Activity of the Root System. Methods of Studying the Absorption of Water by Roots. Absorption of Water by Aerial Parts of the Plant.—One of the environmental factors that strongly

influences the rate of absorption of water by the roots is the temperature of the soil. This was detected long ago by Sachs, by means of very simple and illustrative experiments. He noticed that such potted plants as tobacco, beans, or pumpkin rapidly show signs of wilting when the pot is surrounded with ice. When this is removed and the soil is heated, the plants recover without additional moisture. From this he drew the conclusion that under the influence of low temperature the rate of root absorption slows down so much that it is no longer able to make good the loss sustained by evaporation. Such a simple method,

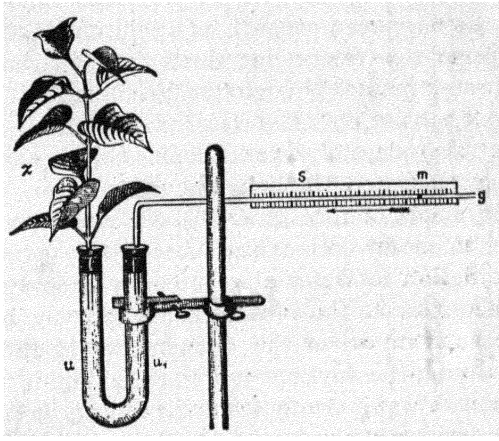


FIG. 89.—Potometer (*after Molisch*).

however, is not sufficient for the exact determination of the amount of water absorbed by the plant. A special apparatus, called the "potometer," is now used, which allows a quantitative determination of the volume of water absorbed (Fig. 89). The essential parts of a potometer are: (1) a container, through the upper end of which a plant, inserted in a stopper, is introduced in such a way that the root system is within the container; (2) a graduated capillary tube, placed horizontally, for recording the amount of water absorbed. A potometer admits of an almost endless number of modifications. Frequently, only branches instead of whole plants are employed. It is often expedient to construct a potometer that can be weighed, in order to be able to compare the water absorbed with that lost by the plant in the process of transpiration.

Exact quantitative determinations that have been carried out by means of potometers have shown that at a lowered temperature the rate of water absorption by the roots rapidly slows down. This reduction, however, is not equal in different plants. The thermophilous plants of the tropics and subtropics show a reduction in their absorbing capacity more rapidly than do plants of temperate zones. The reduced absorption caused by low temperatures is induced not so much by the slowing down of the diffusion rate, which changes but slightly under the influence of temperature, as by the altered properties of the protoplasm. At low temperatures, the viscosity of the fluid protoplasm increases. It may even congeal, as a solution of gelatin does at room temperature. Congealing slows down markedly the rate at which water passes through the protoplasm. Experiments have shown that not only the water absorption of a whole root system, but also plasmolysis and deplasmolysis connected with the passing of water through the protoplasm, are strongly retarded by a low temperature. At 0°C., this process is only one-quarter to one-seventh as fast as at 20°C.

The retardation in water absorption by a cold soil explains many peculiarities in the life of plants growing in temperate zones. In autumn, when the temperature of the air is still quite high during the daytime and transpiration is considerable, the roots absorb water slowly from the already cooled soil. As a result, a considerable water deficit may exist within the plant. The usual response by a plant to these conditions is a reduction of its evaporating surface. This is effected by abscission of leaves through the formation of an abscission layer across the petiole (see Art. 103). According to Schimper, cold soil is physiologically dry, though it may be saturated with water. This explains many peculiar structures in bog plants, which evidently lessen transpiration, since bog soils are cold and thaw slowly in the spring. Many bog plants, like *Oxycoccus*, *Andromeda*, and *Ledum*, have leathery leaves with somewhat involute margins. Their stomata, through which water is given off, are depressed. This structure helps to decrease transpiration. Other bog plants, for instance, *Eriophorum*, have very narrow, almost awl-shaped leaves.

The influence of a low soil temperature on the absorption of water by the plant, however, must not be overestimated. In

hardy plants, as for instance in winter cereals, low temperatures interfere less with the intake of water. These plants grow and develop perfectly well in late fall and early spring, in spite of periodic frosts. Their unhindered absorption of water from the soil is indicated by an abundant guttation.

Oxygen of the soil air is another external factor influencing the rate of water absorption by the root. In order to develop exudation pressure and to absorb mineral salts, as well as for their growth, roots require a supply of oxygen. On too compact or submerged soils, therefore, plants develop poorly or die early. This is observed frequently in spring in small depressions of the ground where water stagnates. It is not the excess of water itself that is injurious to the plant, since it develops perfectly in water cultures. It is rather the lack of oxygen resulting from submersion that is harmful. Plants in water cultures develop successfully only when an air current is passed through the solution or the latter is changed often. Experiments with potometers have shown that a delay in absorption results when the water is saturated with carbon dioxide or nitrogen, the oxygen thereby being removed. The inhibiting influence of carbon dioxide is more marked than that of nitrogen, the former apparently being toxic.

The root system is the special organ of water absorption. This does not mean, however, that the other organs of the plant are unable to absorb water. Every unsaturated cell imbibes water if brought into contact with it. Thus leaves, especially, may absorb water, in spite of their cutinized epidermis. Experiments have shown that a dry cuticle is almost impermeable to water. When moistened, it swells and becomes much more permeable.

Leaves moistened by rain or dew are able, therefore, to take up water. The dormant branches and buds of trees likewise absorb water through their surface during thaws and spring rains. This absorption, however, plays no important role in the general water regulation of the plant. The amount of water absorbed always remains small in comparison with the total amount of water lost. Moreover, owing to the moistening of the cuticle, the water absorbed in this way easily evaporates again and in general increases the rate of transpiration. This explains the well-known rule that the watering of plants in direct sunlight must be avoided, as it induces more rapid wilting.

However in many plants, such as wheat and other cereals, the leaves are not moistened by water owing to their wax covering. As has been proved in experiments with spray irrigation, such plants may be watered at any time of the day and independent of the weather conditions. But even with plants sensitive to wetting of the leaves, cautious watering without sprinkling of the leaves does not produce any harmful consequences, even when done during the hot noon hours.

68. The Process of Transpiration, or Evaporation, of Water by the Plant. Its Role in the Life of the Plant. Methods of Studying Transpiration.—Land plants lose considerable amounts of water through evaporation from the surface of their leaves. Evaporation is essentially a physical process in which water changes from a liquid to a gaseous state in an unsaturated atmosphere and then diffuses as vapor into the surrounding space. The mechanism, however, is considerably complicated by a number of anatomical and physiological structures of the evaporating surface. Hence, it must be really considered a physiological process. It leaves a marked effect on many phases of the life of land plants, and it is responsible for a whole series of other related physiological processes. The evaporation of water by the plant is usually designated by the special term "transpiration."

Transpiration is a physical necessity for all terrestrial plants. In instances of great dryness of the air and lack of water in the soil, a continual loss of water may lead to harmful and even fatal consequences. Emphasizing this, some authors (Schwendener, Timiriazev) believe that transpiration is an unavoidable evil. This, however, is not entirely true. Under normal conditions of growth, the loss of water by the leaves is readily replaced from the soil. Moreover, the process of transpiration is very probably the major cause of translocation of water toward the evaporating leaves. Thus "injury" caused by transpiration is really not so great. Also, the absorption and upward translocation of mineral salts are facilitated by the water current that continually passes through the plant. Another favorable consequence of transpiration is the reduction in temperature of the leaves, which enables them to function even in the brightest sunlight without injury. Experiments have shown that the temperature of wilting leaves, whose transpiration is reduced,

is 4 to 6°C. higher than that of turgid ones, a difference that sometimes under conditions of a high temperature may prove fatal to the plant. In greenhouses and hotbeds, where the moist air lessens transpiration, overheating and severe burning of the leaves have been noted. Another physiological result of transpiration has been pointed out by L. A. Ivanov. According to this author, a certain saturation of the plant is required, especially for its blooming and fruiting. This optimum degree of turgor is maintained by transpiration. All this evidence compels one to believe that transpiration is not an unnecessary evil, but that it is a very important physiological process.

One must not suppose, however, that in order to function normally a plant has to transpire as much water as is usually lost under natural conditions. Observations and experiments have shown that this amount greatly exceeds the necessary minimum. Transpiration can often be considerably reduced not only without injury, but even with advantage, to the plant. It has been noted (Art. 58) that the amount of salts absorbed by a plant is by no means proportional to the amount of water transpired, being to a considerable degree an independent process, and following its specific laws. Later it will be shown that the drier the climate and the higher the transpiration, the more water is used for the production of a certain quantity of organic matter. But it is not possible to make a sharp distinction between useful and excessive loss of water. Transpiration therefore represents a unified physiological process.

The methods for the study of transpiration are comparatively simple. They can be divided into three groups:

1. The collection and determination of the evaporated water.
2. The determination of changes in weight of the plant resulting from loss of water during transpiration.
3. The determination of the amount of water absorbed by the plant to compensate for that lost by transpiration.

[1] To determine the amount of water vapor transpired, the plant under investigation is placed in a tight container, made of glass in order not to exclude light, which plays an important role in transpiration. The water transpired is collected by means of some hygroscopic substance, such as dried calcium chloride. The increase in weight of the drying agent shows the amount of water

transpired. In stagnant air, however, transpiration is retarded. It is expedient, therefore, to draw a current of air through the vessel containing the plant and then to pass this air through calcium chloride tubes or an apparatus containing some other water-absorbing substance (Fig. 90). The advantages of this method are that the leaf or branch under investigation remains attached to the plant. This is almost the only method that can be used to determine the transpiration of trees under natural conditions. Its defect is that the transpiring organs are enclosed

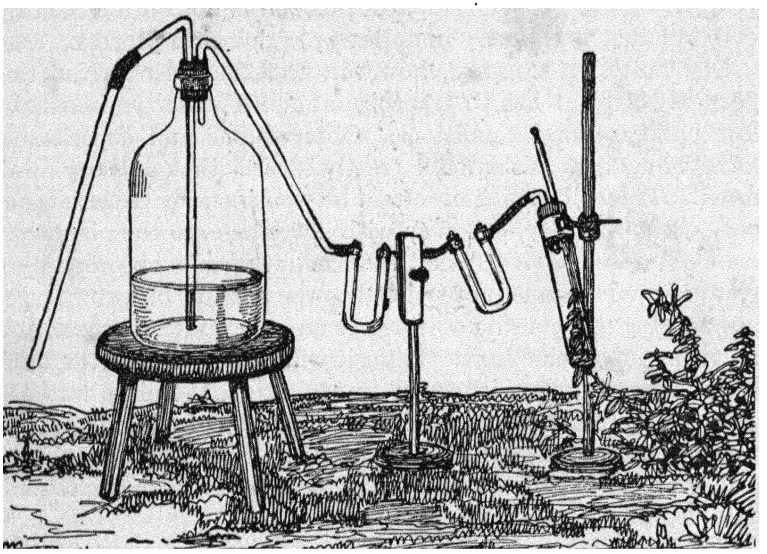


FIG. 90.—Apparatus for the study of transpiration (*redrawn after Freeman*).

in a container, which must undoubtedly interfere with the normal course of transpiration.

Somewhat analogous to the quantitative methods, which are based on the increase in weight of a water-absorbing substance, is the cobalt chloride method, in which the color changes exhibited by filter paper that has been impregnated with a solution of cobalt chloride and dried are used to indicate the transpiration rate. When dry, this paper has a blue color; when moist, it becomes pale pink. If a slip of such paper is placed on the transpiring surface of a leaf and then covered with glass, its color will change (Fig. 91).

The more rapidly this change takes place, the higher the rate of transpiration. By this simple method, it can readily be demonstrated that there is a considerable difference in the rate of water loss from the upper and lower surfaces of leaves that have their stomata mainly or wholly on one surface. The cobalt chloride method is only a qualitative one, though during recent years repeated endeavors have been made to raise it to

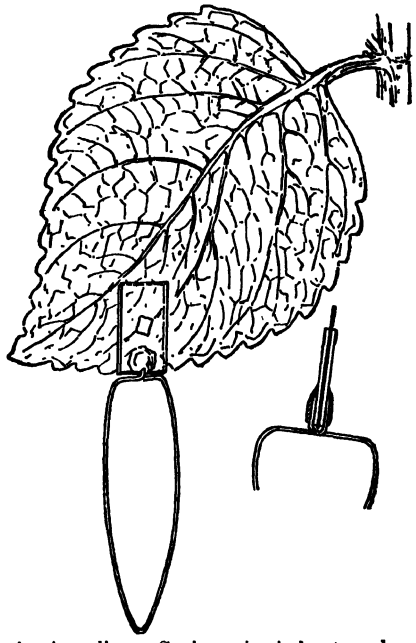


FIG. 91.—Transpiration clips. Spring wire is bent as shown and cemented to small glass slips about 1½ by 2 cm. in size. Two-millimeter squares of cobalt chloride paper are then clipped to the leaves as shown, to measure their relative transpiration rates (*after Loomis and Shull*).

the level of a quantitative method by measuring with a stop watch the time required for the blue paper to turn pink.

The most reliable methods of studying transpiration are those based on changes in weight of the plant resulting from loss of water. It may be supposed that such methods might introduce a considerable error; for changes of weight depend not only on the loss or gain of water, but also on the gain or loss of dry substances, due to assimilation and respiration. Numerous experiments have shown, however, that the weight of water lost

by a plant per unit of time is several hundred times as great as the increase in dry weight. The error resulting from not taking into consideration assimilation and respiration is therefore negligible.

For the study of transpiration, whole plants rooted in soil, or cut branches, or leaves may be used. Rooted plants permit

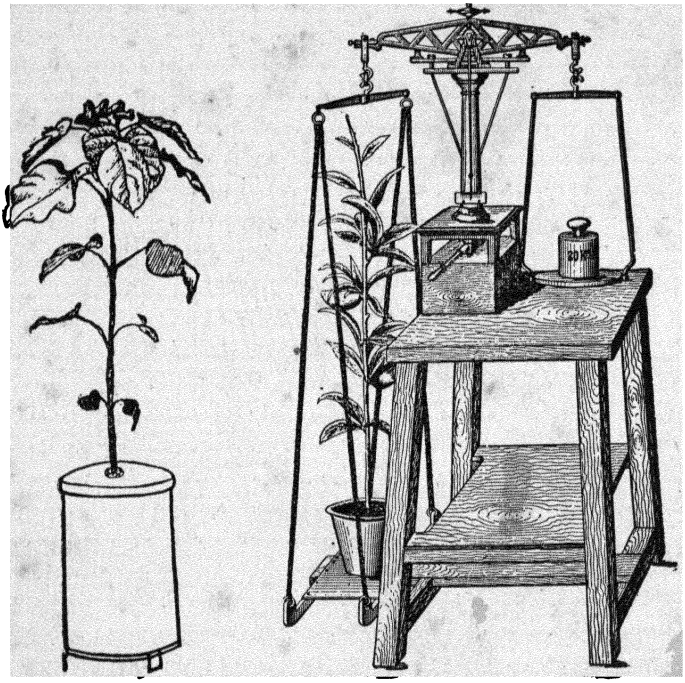


FIG. 92. FIG. 93.

FIG. 92.—Galvanized-iron pot with closely fitting cover for the exact determination of water used by a plant.

FIG. 93.—Transpiration balance (*after Burgerstein*).

the study of transpiration under more natural conditions, but the necessity of weighing such plants together with the soil in which they grow introduces a series of technical difficulties. The plants either have to be grown in special containers impermeable to water vapor (Fig. 92); or, at the time of the experiment at least, the pot must be enclosed in some metal or rubber covering, in order to prevent evaporation from the surface of the soil. For more exact experiments, special transpiration balances (Fig. 93) combining high sensitivity with large capacity have to be used.

Experiments with cut shoots and leaves are more convenient, as there is no necessity of weighing heavy pots of soil, which must inevitably affect the sensitivity of a balance. Besides, no special containers are required for the growing plants. Hence, this method of studying transpiration is widely used. The shoot is placed with its cut end in water, so that the water lost will be continually replaced. Precautions must be taken to prevent the evaporation of water otherwise than through the shoot. This is accomplished by inserting the twig through a hole in the stopper of the container (Fig. 94). Air is permitted to enter the container through a glass tube inserted in the same stopper. Experiments of long duration with cut plants are not very reliable; for the conditions of water absorption are altered, and when placed in direct sunlight, cut portions of a plant may wilt rapidly. Frequently, cut leaves and shoots transpire more rapidly, when placed in water, than do those remaining on the plant, since they may have a better water supply. That is why more reliable results are obtained if the cut parts of a plant are immediately weighed upon cutting and the weighing is repeated at short time intervals of 1 to 2 min. The cutting is best carried out under melted paraffin in order to preclude the penetration of air into the vessels from without, as well as the desiccation of the surface of the cut. This method may give fairly exact data on the transpiration of plants in the natural conditions of their habitat.

The determination of transpiration by measuring the volume of water absorbed is carried out by means of various potometers, which have already been discussed in Art. 67. Potometers are useful for purposes of demonstration. They also have the advantage that the readings may be taken uninterruptedly. But the amount of water absorbed does not always coincide with the amount of water transpired. Even during a limited period, the error may be as high as 50 per cent. In exact experiments, therefore, the gravimetric method of determining transpiration is to be preferred to the volumetric.

In order to obtain a continuous record of the progress of transpiration, self-recording mechanisms called "transpirographs" are used. In the majority of cases, these are transpiration balances in which the movements of the hands or the scales are transmitted to a revolving drum with a smoked surface.

Experiments with transpirographs are usually successful only in a closed room, such as a greenhouse. When they are conducted out of doors, wind interferes with the accuracy of the records.

In order to compare the results obtained by different experiments, it is necessary to reduce them to a common standard unit. Most frequently, the total quantity of water lost by a plant during a certain period is expressed per unit of its leaf surface. The magnitude thus obtained is called the "intensity of transpiration" and is usually expressed in grams per hour per square meter of leaf area. Sometimes, instead of the use of the surface area, which in highly dissected leaves of irregular shape is difficult to determine, the green or even the dry weight of the leaves is used in calculation. In comparing the amount of water lost per unit time with the total amount of water contained in the plant, the *rate of expenditure of the water reserve*, expressed in percentage, is obtained. In comparing the amount of water lost by the plant during a rather long period, for instance, several weeks or even the entire growth period, with the amount of dry matter accumulated in this time, the *transpiration efficiency*, expressed in grams per kilogram of water lost, is obtained. The reverse value, the number of grams of water used in accumulating 1 g. of dry substance, is termed the "transpiration coefficient." Some authors, chiefly American, call this value the "water requirement" of the plant.

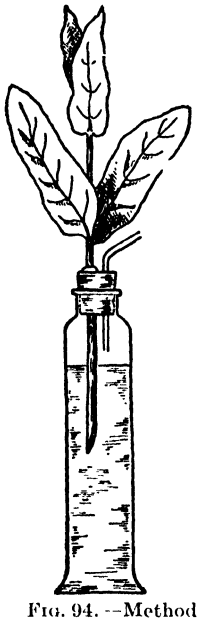


FIG. 94.—Method of determining the water used by a shoot.

An idea of the intensity of transpiration is obtained by comparing the rate of transpiration from a unit leaf surface with the rate of evaporation from a unit area of a free water surface. The magnitude obtained is called "relative transpiration." It shows to what extent the transpiration of a leaf is slower when compared with "free evaporation."

All these magnitudes vary greatly in different plants and under the influence of different environmental conditions. In most plants, the intensity of transpiration ranges from 15 to 250 g. per square meter per hour in daytime, and from 1 to 20 g. at

night. The rate of water expenditure may fluctuate from 10 to 80 per cent; the efficiency of transpiration, from 1 to 8. Under the conditions of a moderately moist climate, it is frequently 3. The transpiration coefficient correspondingly varies from 1,000 to 125. Most often, it is 300. Relative transpiration is commonly expressed by values of 0.1 to 0.5, sometimes almost attaining 1, and in other cases falling to 0.01 or below, especially in plants well protected from water loss.

69. The Physical Aspects of Transpiration. Its Dependence on Environmental Conditions and Its Daily Changes.—Essentially, transpiration represents the physical process of evaporation and as such may be expressed by Dalton's formula,

$$V = K(F - f)\frac{760}{P}S,$$

where K is the coefficient of diffusion or evaporation; F, the saturation pressure of water vapor in air at the temperature of the evaporating surface; f, the observed water vapor pressure in the surrounding space; P, the barometric pressure; and S, the area of the evaporating surface. From this formula, it follows in the first place that transpiration is proportional to the saturation deficit of the air, not to the relative humidity as is often erroneously stated. Second, the formula shows that with a rise in temperature the rate of transpiration rises; for then the value of F increases, and very rapidly.

Evaporation from areas of small dimensions shows considerable deviations from Dalton's rule. According to Stefan's law, referred to in Art. 44, evaporation from a small surface is proportional not to its area, but to its diameter. Other conditions being equal, several small leaves lose more water than a large one of equal area. The greatest deviations from Dalton's law may be observed when the air is perfectly still, which is rarely realized under natural conditions. As soon as wind comes into play, these differences become considerably smaller. In general, wind markedly increases evaporation and consequently transpiration, by removing the moist air from the surface of the leaf and replacing it with dryer air. Wind likewise decreases slightly the atmospheric pressure on the lee side of the leaves, thus drawing the moist air from the intercellular spaces within the leaf.

There is no direct proportionality between the velocity of the wind and the magnitude of transpiration. A strong wind increases transpiration not much more than a breeze does; for evaporation, *i.e.*, the change of water from a liquid to the gaseous state, actually occurs in the intercellular spaces of the leaf, which are protected from the direct influence of the wind.

Light has a very important function in the process of transpiration. Since chlorophyll absorbs certain wave lengths of radiant energy, the temperature of the leaf is increased perceptibly in light. This immediately increases the difference between the factors F and f and consequently intensifies transpiration, which in turn lowers the temperature of the leaf. Hence, plants that have a high power of evaporation are heated hardly at all in light, but their transpiration increases considerably. It was stated in the chapter on assimilation (Art. 42) that usually only 1 to 5 per cent of the radiant energy absorbed by the plant is used in photosynthesis, while over 80 per cent is spent in transpiration. Even diffuse light increases transpiration 30 to 40 per cent, while in direct sunlight it may be several times greater. This accounts for the marked difference between day and night transpiration, which is many times greater than differences in evaporation produced by any other factors.

Besides the direct heating effect, light increases transpiration indirectly also. It favors the opening of stomata and increases the water permeability of the protoplasm of the evaporating cells. Both of these circumstances favor the loss of water from plants.

The influence of environmental factors controls the daily changes in transpiration. Transpiration is low in the early morning hours and increases rapidly with the rise of the sun, which is followed by a rise in temperature and an increase of the saturation deficit. It attains its maximum in the early afternoon and again falls rapidly with the setting of the sun. If curves are plotted for the diurnal changes in transpiration, solar radiation, the saturation deficit, and temperature, it will be noted that these curves will almost coincide. Radiation, however, reaches its maximum slightly ahead of transpiration, while the highest point in the saturation deficit is attained somewhat later (Fig. 95). This shows that sunlight is the chief factor determining the intensity of transpiration.

A typical daily trend in transpiration is naturally observed only on absolutely cloudless days, when meteorological conditions are normal. It will show sharp fluctuations in changeable weather, largely according to alterations in temperature and light.

70. Leaf Structure as a Factor in Transpiration. Stomatal and Cuticular Transpiration.—The rate of transpiration is deter-

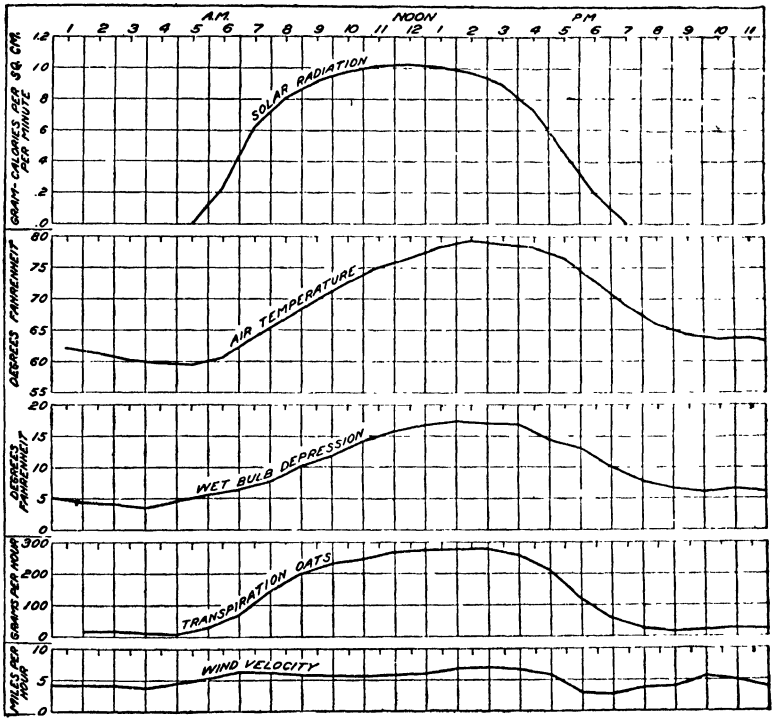


FIG. 95.—Graphs showing the daily march of transpiration and the most important factors determining it (*according to Briggs and Shantz*).

mined not only by external factors, but to a high degree likewise by the structure of the leaf, as well as the state of its cells and tissues during transpiration. These factors complicate considerably the process of water loss.

Evaporation proper, *i.e.*, the transition of water from a liquid to a vaporized condition, takes place at the surface of the parenchymatous cells lining the intercellular spaces of the leaf. These open spaces represent a special aeration system, the outlets of which are the stomata. The rest of the leaf is covered by a solid

layer of epidermal cells, whose outer walls are coated with a cuticle that is only slightly permeable to water or water vapor (see Figs. 54 and 102).

The cuticle, not being entirely impermeable to water, does not completely stop evaporation. This can be demonstrated by coating with vaseline the lower surface of leaves that have no stomata on the upper surface; there are many trees the leaves of which are well suited to this purpose. These leaves will continue to transpire, although at a reduced rate. This loss of water through the cuticle is called "cuticular transpiration," as distinguished from "stomatal transpiration," in which water escapes through the stomata. In fully developed leaves, stomatal transpiration is ten to twenty times as rapid as cuticular transpiration. In young leaves, with cuticle that is not completely developed, as well as in leaves growing in the shade or in a moist atmosphere, half of the water may be transpired through the cuticle.

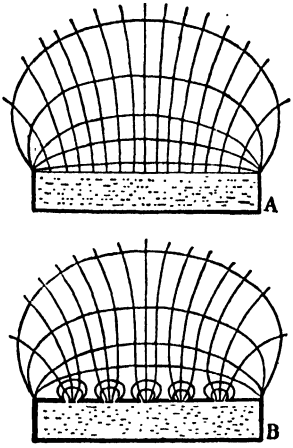


Fig. 96.—Diagrams showing diffusion of water vapor from an open vessel *A* and through a multiperforated membrane *B* (*after H. Walter*).

Stomatal transpiration consists of two phases: the evaporation from the surface of the water-saturated mesophyll cells lining the intercellular spaces, and the diffusion of the vapor thus formed through the stomata. The laws governing diffusion through stomata have been discussed previously. It has been shown that the great number of these very small openings in the cuticle strongly favors diffusion. This process may go on through the stomatal openings, constituting only 1 to 2 per cent of the leaf area, almost at the same rate as if the cuticle did not exist and the internal cavities were fully open to the air. A diagram illustrating this is shown in Fig. 96. In some very vigorously evaporating plants, the relative transpiration may attain the magnitude of 0.8 to 0.9; while under favorable conditions, 0.4 to 0.5 are average figures. If transpiration were proportional to the area of the openings, then the values would not exceed 0.01 to 0.02. Thus it is seen that, per stoma, the diffusion rate of water vapor is very high.

The diffusion capacity of the epidermis supplied with innumerable stomata is so high that the plant seldom uses it to full capacity. This is true at least for the process of transpiration. The stomata play also an important part in assimilation, as they are the openings that give access to carbon dioxide. Actually, transpiration is always considerably lower than its calculated value. From this, the conclusion may be drawn that besides changes in the stomatal openings, other factors may exert a checking influence on transpiration.

71. Stomatal Apparatus of the Plant. Its Movements and Methods of Its Study. Nonstomatal Regulation of Transpiration.—One of the most important peculiarities of the stomata is their ability to change the diameter of their opening. Details of the anatomical structure of the stomatal apparatus are given in books on plant anatomy and will not be discussed here. It will only be indicated that the stomatal opening is limited by two guard cells of a beanlike shape (Fig. 97). The internal walls of these cells lining the opening are considerably thickened, while the outer walls remain thin. Unequal thickness of the outer and inner walls of the guard cells determines the possibility of changes in the diameter of the opening. When the guard cells imbibe water, their outer walls are much more expanded than their inner ones; this leads to a curvature of the cells, and the opening increases. Conversely, with loss of water the cells decrease in volume, their walls straighten, and the opening closes.

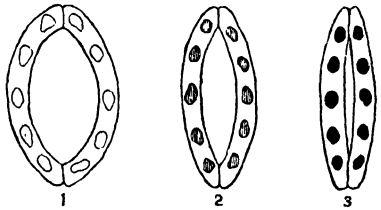


FIG. 97.—Changes in the starch content of guard cells during their movements (*after Iljin*).

Light and water content are the chief factors responsible for the movements of the guard cells. With abundance of water in the leaf, the guard cells are saturated with water, the cell walls are extended, and the stomata are open. Deficiency of water leads to a decrease of turgor and to a closing of the stomata. In the majority of plants, the stomata are opened in light and closed in darkness.

The chemical processes causing the changes in turgor of the guard cells and thus controlling stomatal movements are very

peculiar and not yet fully understood in all their details. Transformation of starch into sugar occurs in the guard cells, and this is regulated by enzymatic processes. When starch is transformed into sugar, the sap concentration, and consequently the suction tension of the cell, increases. When guard cells imbibe water, their volume and their curvature increase, resulting in their separation and a widening of the opening between them. But when sugar changes into starch, the reverse processes take place, and the stomata close (Fig. 97). This connection between the movements of stomata and the transformations of starch has been established by Iljin. It is of interest to note that the influence of external factors on the transformation of starch in the guard cells and in other cells of the leaf is very different. In the mesophyll cells, starch is formed in light and transformed to sugars in darkness. In guard cells, on the contrary, starch disappears in light and is again accumulated in darkness. Lack of water leads to the transformation of sugar into starch, and the stomata close. With a sufficient amount of water in the cell sap, sugar accumulates, and the stomata are opened.

Since stomatal movements are controlled by a complex mechanism, their response to changes in the environment is also quite complex. Under extreme conditions, such as very severe wilting or a rise of temperature above 40°C., as well as under the action of sodium and potassium, the mechanism undergoes striking disturbances. The hydrolysis of starch then rises rapidly, while the converse process, the transformation of sugar into starch, is entirely suppressed. This causes a great increase in osmotic pressure of the guard cells. They become saturated with water, open abnormally wide, and often lose the capacity to close again. Under unusual dryness of the air, this may lead to a complete desiccation of the leaf. According to the investigations of Zalensky, such abnormal opening of the stomata is observed during hot desiccating winds, which explains their harmful effect.

The mechanism of stomata may be studied in various ways. The simplest method would be the direct observation under the microscope of stomatal movements in an uninjured leaf. But on account of their small size and the lack of transparency of the leaves, this method can be used only with a few plants. Moreover, it is inconvenient to work with a microscope in a field or

forest. The method proposed by Lloyd is therefore used more frequently. According to this procedure, a strip of epidermis is quickly removed with forceps and plunged into absolute alcohol. This instantly dehydrates the cell walls and preserves them in the condition they were in at the moment of stripping. Such a fixed preparation may be kept indefinitely and examined under the microscope whenever necessary.

A still simpler method was devised by Molisch. It is based on the fact that liquids capable of wetting the cuticle will readily penetrate the open stomata and rapidly fill the intercellular spaces of the leaf. Such an injected leaf becomes quite transparent. In order to determine the degree of opening of stomata, a drop of benzene or absolute alcohol is placed on the leaf by means of a small brush or glass rod. If the stomata are open, the drop is rapidly absorbed, and transparent patches appear on the leaf (Fig. 98). If they are closed, the drop will dry rapidly without leaving any trace. As benzene can penetrate into narrower openings than alcohol, it may infiltrate a leaf with nearly closed stomata. By applying both liquids alternately, it is possible to determine not only whether the stomata are open or closed, but also the approximate degree of opening. Instead of benzene and alcohol, other liquids, such as petroleum ether or kerosene, may be used. The infiltration method is not exact, but it is simple and well adapted for use under natural conditions.

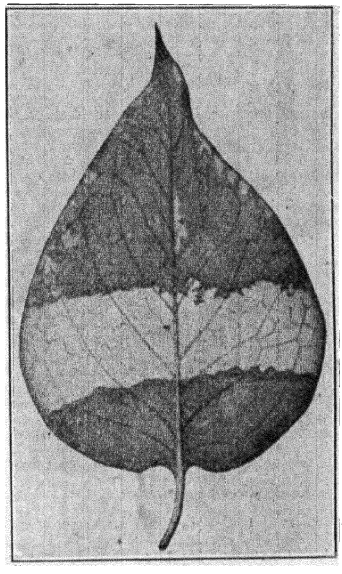


FIG. 98.—Transparent band formed after the infiltration with benzol across a part of a leaf that was illuminated and therefore had open stomata (*after Molisch*).

For determining the degree of opening of the stomata, Francis Darwin devised a special apparatus called by him the "porometer." It consists of two parts: a bell jar about 1 cm. in diameter, and a T-shaped tube (Fig. 99). The tiny bell jar is glued by its flange to the leaf surface. This leaf chamber is connected by a rubber tube with one of the horizontal arms of the

T-tube. The other arm ends in a rubber tube, which is controlled by a clamp, while the vertical part of the tube dips into a vessel of water. When air is sucked through the rubber tube and the clamp then closed, the air within the tube and bell jar becomes rarefied, and the water is raised in the vertical arm. If the stomata are open, the air enters through them into the bell jar, and the water column in the tube falls. The rate of falling indicates to some extent the degree of opening of the stomata. When they are completely closed, the water column remains stationary for many hours at the height to which it was raised.

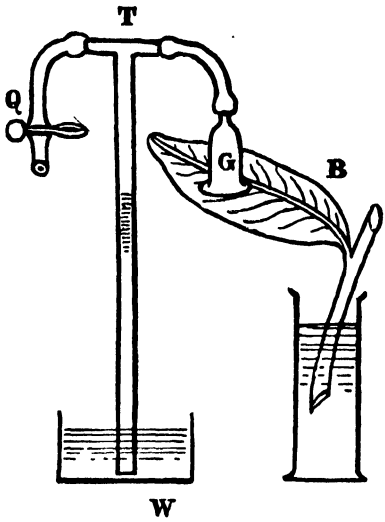


FIG. 99.—Rate of fall of water *W* in tube *T* indicates rates of passage of air into bell glass *G* through leaf *B* (*after F. Darwin*).

By using any of the described methods, the daily changes of the stomatal movements may be observed. It has been noted that in bright but not too dry or hot weather, there are fairly regular diurnal changes. In the majority of plants, the stomata open at dawn, or even earlier. The maximum opening is usually attained in the morning hours. About noon, the stomatal slit begins to narrow slightly, and it closes usually a little before sunset. When the sky is overcast, the openings are not so wide as in bright light. In very dry weather, the slits open in the morning but soon close again as a result of loss of water by the leaf. Frequently about noon, or between ten and eleven o'clock in the morning, the stomata may have shut completely. Under conditions of maximum heat and dryness, they remain closed the whole day long and open only for a short time during the early morning hours.

The behavior of stomata varies with different plants. In some, like the potato, cabbage, beet, etc., they are usually open, and under optimum conditions of water supply they tend to remain so, throughout the day and night. In other plants,

for instance, the cereals, stomata are always shut at night. Moreover, they close very early in the evening and at the slightest deficiency of moisture may be closed even in the morning hours. In the majority of plants, the behavior of stomata can be said to be between these extremes (Fig. 100). It is interesting to

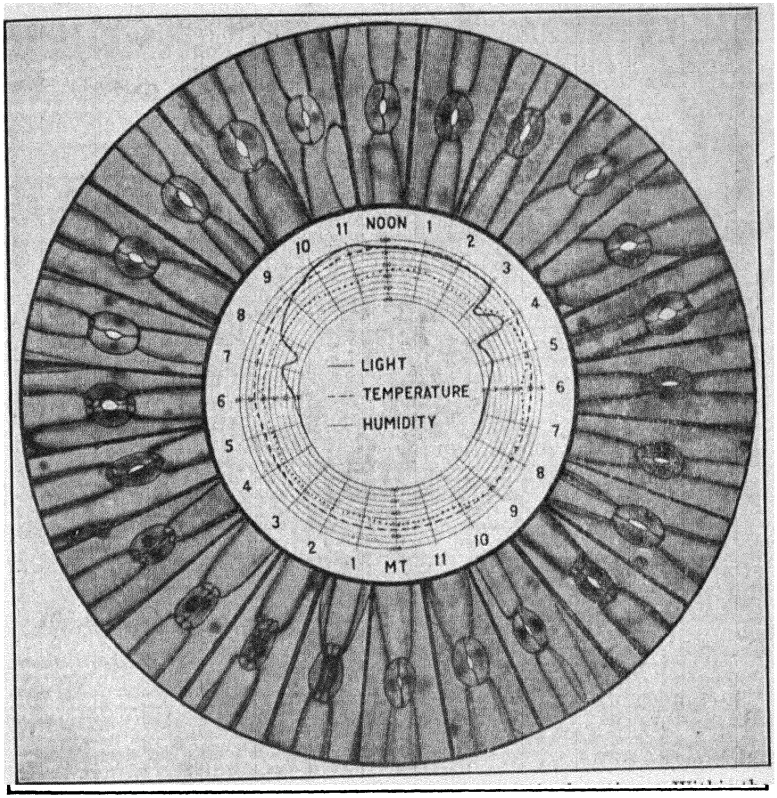


FIG. 100.—Daily changes in the stomatal movements in the onion. Within the circle are graphs showing diurnal changes in light intensity, temperature, and humidity (*after Loftfield*).

note that in plants showing stomata on both sides of the leaf, those on the upper surface open later and for a shorter time, than those on the lower surface. Likewise, the stomata near the tip of the leaf open earlier and close later than those at the base.

Since changes in the degree of opening of the stomata may be observed directly, the stomatal movements until recently were regarded as the only means of regulating transpiration. This

controlling influence of the stomata is based on the fact that when the stomata are closed, outward diffusion of water vapor is entirely stopped. The air in the intercellular spaces then becomes saturated, and water is no longer evaporated; thus there can take place only cuticular transpiration, which is comparatively insignificant. There results a considerable decrease in the total water loss. The marked reduction in transpiration at night, mentioned in Art. 69, depends not only on environmental conditions, but also on the fact that in the majority of plants the stomata are completely closed at night.

But the fact that the complete shutting of stomata stops transpiration does not permit the conclusion that a mere narrowing of the stomatal pore will lead to a corresponding check in water loss. The epidermis with its stomata may be viewed as a multiperforated septum. On account of the small dimensions and great number of these openings, diffusion takes place almost at the same rate as if a septum did not exist (Arts. 44 and 70). Within certain limits, changes in the stomatal pores therefore do not always induce a slowing down of the diffusion rate. An exact correlation between the degree of opening of the stomata and the intensity of transpiration has not yet been established. Recent investigations, however, make it very probable that a decrease of 50 to 75 per cent in the diameter of a stomatal pore does not seem to influence transpiration very greatly. A further narrowing of openings, however, will result in a perceptible check in water loss.

In estimating the regulatory role of the stomata, it must be remembered that light, which so greatly influences transpiration, promotes the opening of the stomata. They close in light only with great difficulty. This influence of light on the movements of stomata may seem incomprehensible as regards water economy. It is understandable, however, when it is remembered that the stomata are the channels through which carbon dioxide must enter the leaf; and that for successful photosynthesis, it is requisite that the stomata be open during the hours when the plant is illuminated by the sun. The closing of the stomata during the daytime, though lessening transpiration, would have an unfavorable effect on the nutrition of the plant.

Another way of regulating transpiration would be by a reduction of evaporation from the surface of the cell walls within the

intercellular spaces. Such decrease can take place only when the cell walls lining the intercellular spaces become drier than usual. Such a dehydration of the walls in turn is subject to a lower degree of saturation of the cells themselves and, consequently, an increase in their suction tension. As a result of this mechanism, based on the equilibrium between the suction tension of the cell contents and the colloidal cell walls, a water shortage in the leaf, due to excessive transpiration or an insufficient water supply from the soil, is in itself a cause of decreased transpiration, independent of the degree of opening of the stomatal pores. This phenomenon has been called "incipient drying" (Livingston). It must be regarded as a most important nonstomatal regulator of transpiration.

72. Water Balance of Plants. The Phenomenon of Wilting and Its Significance.—Under usual conditions, the transpiration of plants is fairly well coordinated with the absorption of water. The relation between transpiration and absorption, or the water balance, is favorable for growth showing neither excess of water nor any considerable deficit. The water content of plants, expressed in percentage of the dry weight, represents under usual conditions a fairly constant magnitude.

An increase in transpiration such as may be observed during weather with bright sunshine may disturb this equilibrium and cause a water deficit, up to 5 or 10 per cent, even when the soil moisture is sufficient. With lack of soil moisture, this deficit may increase up to 25 per cent and more. A water deficit occurring mostly in the afternoon represents quite a normal phenomenon and is not injurious to the plant. Its excessive increase is inhibited by the ability of the plant to regulate its transpiration losses within a fairly wide range and even under a high rate of water loss.

The plant can regulate its transpiration only within certain limits and with a considerable increase of transpiration; or with drying of the soil, when absorption of water is inhibited, the water balance is disturbed significantly. This becomes evident in the phenomenon of wilting. Wilting is the result of a loss of turgor, the tissues losing the turgor pressure proper to them in a state of saturation and the leaves and young stem tips drooping down. Wilting, however, does not indicate a loss of vital activity. If the plant is provided in time with water,

turgidity is reestablished, and the plant resumes its normal vital activity.

There may be distinguished two types of wilting, temporary and permanent. The first may be observed in cases when excessive heat and dryness of the atmosphere cause such an increase in transpiration that water is absorbed from the soil too slowly to compensate for expenditure. In this case, loss of turgor and wilting are displayed by the organs that lose water most rapidly, such as leaves, while the other organs of the plant remain turgid. As soon as transpiration is decreased, for instance, toward the evening, the water deficit decreases, and the plant recovers without any additional moistening of the soil. Temporary wilting is not so very injurious to plants; however, it may decrease the yield, for during wilting, photosynthesis and growth are retarded. This may often be observed with sunflowers and sugar beets, pumpkins, and other agricultural plants.

Permanent wilting takes place when the soil does not contain any more water available to the plant. Under these conditions, transpiration, however weak it may be, leads to a gradual loss of turgor in all the organs of the plant down to the root hairs. The root hairs apparently are very sensitive to a lack of water and die quickly. This leads to a disturbance in the contact that existed between the root and the soil. This is the reason why plants, when abundantly watered after permanent wilting, absorb water very slowly; only when new root hairs have formed, is the usual rate of water absorption reestablished. Furthermore, the withdrawal of water from the chlorophyll-bearing cells injures the chloroplasts, which for a long time decrease or even completely lose their photosynthetic ability. For this reason, wilting is very harmful to the nutrition of plants.

Permanent wilting is no less injurious to other organs and tissues. It retards the growth of the young organs. If wilting occurs in the beginning of seed formation, the filling and ripening of grain are irregular, the yield decreases, and the grain is of low quality. This phenomenon is sometimes observed, also, after temporary wilting, though to a lesser degree.

Wilting, especially permanent wilting, is definitely harmful to plants. Experiments show that it is accompanied by a very sharp decrease in the water loss, as a consequence of the closing of stomata as well as of the incipient drying of the cell walls.

In a wilted state, the plant transpires from one-fifth to one-tenth of the water lost when in a turgid state. That is why wilting must be regarded as one of the most effective means of inhibiting transpiration in critical periods of the life of the plant.

Various plants show signs of wilting, or of loss of turgor, at varying degrees of water loss. Those of open sunny habitats, as for instance the sunflower or potatoes, may lose up to 25 or even 30 per cent of their total water content without displaying any signs of wilting. Such a water deficit may usually be observed in these plants at noon on a hot day. Other plants, chiefly shade plants, wilt with a loss of only 2 to 3 per cent of their water reserve. This difference is determined by the fact that in the first group of plants the cell wall is very distended, like a rubber balloon, and retains a certain degree of elasticity even with a considerable decrease in volume. In the second group, the cell walls are under tension but not distended; consequently, a small decrease in volume leads to collapse of the cell walls.

The capacity of losing a considerable amount of water without wilting has the advantage of allowing the stomata to remain open, the plant being thus able to continue photosynthesis.

Besides decreasing the water loss, wilting leads to other deeper internal changes, which finally result in an increase of the capacity of the plants to withstand further harmful influences of drought. These changes are known as "drought hardening." They will be further described during the discussion on the drought resistance of plants. In drought hardening, the plant increases its capacity for photosynthesis, transpiration, and other physiological functions (Tumanov, 1926). In the cultivation of crop plants in arid regions, hardening of the plants in their early growth stages is essential. In regions where moisture is generally adequate, it is desirable to avoid the hardening of plants such as tomatoes. If such plants are subjected to hardening treatment, their growth is checked, and the stems become hard and woody; and later, when their roots become established, there is insufficient conduction through the wood to give a vigorous growth. Hardened plants are stunted, whereas plants kept always in rapid growth continue to grow rapidly and bear fruit quicker than hardened plants.

Many plants, such as celery and cabbage, when exposed in seedbeds to low temperature for short periods, will later "shoot to seed" and become unmarketable.

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CHAPTER IX

TRANSLOCATION OF SUBSTANCES IN THE PLANT

73. General Concept of the Ascent and Descent of Water in the Plant.—The plant consists of diverse organs, situated in physically widely differing conditions and having distinct functions. The leaves elaborate organic substances and are in need of water supply and mineral salts; the roots absorb water and mineral substances but require for their respiration a supply of organic substances requisite for absorption and for their further growth; flowers, fruit, and growing tips rapidly consume organic substances, and so there must be a continuous supply from the leaves. A considerable part of the photosynthate is stored by the plant; for its synthetic activity is interrupted at night and during cold and dry periods of the year. For these periods of checked activity as well as for the regrowth of its deciduous organs, it requires a store of all the organic substances necessary for the maintenance of its vital processes and for the formation of the cells. Lacking this, some of the older organs, especially leaves, are destroyed to release their "food" material.

This translocation of substances is elaborately developed in woody plants, which often reach very large dimensions. Trees serve as the first objects for the study of the mechanism of translocation and of its basic laws.

The first exact information concerning the translocation of substances was obtained by the Italian scientist Malpighi in 1670 by means of ringing of the bark. Soon after this, the knowledge of the transport mechanism was considerably extended by the classical investigations of the English scientist Hales (1727).

These first physiological experiments were later repeated by other investigators with more exact methods. Experiments have shown that the translocation of substances in the tree trunk proceeds in two directions. One stream ascends from the roots to the leaves and carries water and mineral elements. As the general direction of this movement is upward, it is termed the

"ascending stream," though in particular cases, for instance, in drooping branches, it may move downward. At any rate, its chief direction is from the base to the tip of any organ of the plant. The other stream carries chiefly organic substances. It originates in the leaves, which elaborate these substances, and is directed mainly downward along the stem to the roots, where these substances are utilized in growth and respiration. That is why it is termed the "descending stream." But its direction is not so definite as that of the ascending stream. It may also move upward from the leaves to the growing points, to branch ends, blossoms, and developing fruit. That is why it is frequently termed the "stream of organic, or food, substances" without reference to its direction.

The existence of these two streams in the plant may be demonstrated by the classical method of ringing, which may be done in the following manner. Cuts one above the other are made completely through the bark and around the trunk or branch, and the bark between them is removed. Care must be taken to remove all of the bark without injuring the young tender outer layers of wood. To protect the exposed wood from desiccation, it is covered either with thin rubber or with wax paper, or it is coated with liquid wax. Leaves above the trunk treated in this way remain alive and do not show any signs of wilting. Growth of the flower buds and fruit proceeds unimpeded. On ringed large branches, the fruit sometimes even reaches unusual size and quality. This proves that the absorption of water and mineral substances proceeds quite normally, and consequently that the ascending stream moves along the wood.

The descending stream, moving through the bark, is stopped by the ringing. Nutritive substances accumulate at the upper edge of the cut, resulting in an excessive outgrowth of the tissues of the bark, forming a callus, which grows over the denuded wood (Fig. 101). If the ringed region is not broad, this callus may reach down to the lower edge and join with the lower bark. The wound then heals, and the downward movement of the sap is resumed. But if the ring is broad enough and not a single branch but the main trunk is ringed, the roots cease to obtain nutritive substances from the leaves and gradually die from starvation. This interrupts water absorption, the ascending stream is likewise stopped, and finally the ringed tree dies.

The organic substances move along the sieve tubes in the phloem cells of the conductive tissue of the bark. This can be demonstrated by ringing experiments with woody plants; for in these, there is a complete ring of xylem, transporting water and surrounded by a complete ring of phloem, representing the conductive part of the bark. It is interesting to note that plants possessing sieve tubes both outside and inside of the xylem ring (as for instance the oleander) do not give clear-cut results in ringing experiments, for translocation of organic substances continues through this internal phloem after the ringing.



FIG. 101.—A branch showing development of callus at the upper edge of the ringed region (*after Brown*).

In some instances, the ascending stream may carry organic substances. This occurs during the spring, when the sap flows and the reserve substances stored in autumn, in the wood and bark of the roots and stems, are digested rapidly and, penetrating in large quantities into the vessels of the wood, are carried upward to the opening buds under the influence of root pressure, which is very intense at this time of the year. At other seasons, the vessels of the wood contain very little organic substance, which, as has been said, is carried principally through the sieve tubes.

The ascending transpiration stream moves through the conducting vessels of the wood, which are formed by vertical rows of dead cells that have lost their contents. These form continuous tubes of capillary dimensions by being joined through pits or reticulate openings through their end walls. Sieve tubes, through which the stream of organic substances is translocated, are elongated living cells containing protoplasm and provided with multiperforate transverse walls, similar to a sieve. The mechanism of transport and forces that move the streams are entirely different in xylem and in phloem, and they must be discussed separately.

74. General Nature of Water Translocation in the Plant. Osmotic Movement of Water from One Cell to Another.—From both a physiological and structural point of view, the course of water movement in the plant may be divided into two parts. Water moves mainly through the vascular system of the plant,

which consists of dead cells, such as tracheids and vessels, representing as it were water-pipe lines. In herbaceous plants, the length of this path may be several centimeters, while, in trees, water may move in this manner hundreds of feet. The second part of the water-translocation system consists of living cells. Through these, water moves for only short distances of a few millimeters or less. There are two small layers of tissues thus traversed by water: one in the root, from the surface of the root hair to the vessels in the central cylinder; the other in the leaf, from the vessels of the fibrovascular bundles to the mesophyll cells bordering the intercellular spaces. The course taken by the water current through these tissues is represented diagram-

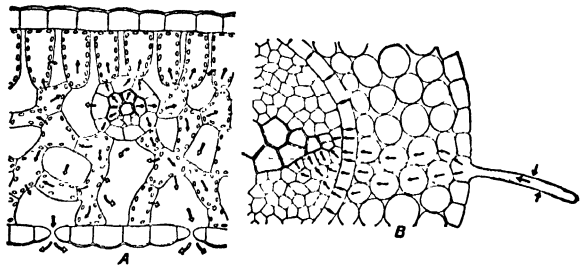


FIG. 102.—Diagram showing the course taken by water current in the plant The black arrows show movement of water in liquid form; the light arrows, that of water vapor (*after Smith, et al.*).

matically in Fig. 102. On the right is shown a root hair transmitting water through a number of parenchyma root cells. Having passed to the endoderm and pericycle, it enters the vessels of the central cylinder, through which it is moved to the leaf. Here it passes again through a number of parenchyma cells and is finally evaporated into the atmosphere.

These few millimeters of translocation in the living cells are subject to greater difficulties than are the many meters traversed in the conductive tissues. The ease with which water may be moved through the vessels and its slow movement from cell to cell may be seen from the following simple experiment. When a succulent leafy stem with numerous parenchyma cells, for instance, the stem of the balsam plant, is cut and placed in water, it will remain fresh for a long time, as the water readily enters through the cut vessels and ascends to the leaves. When the cavities of the vessels are sealed up by immersing the cut

end in liquefied gelatin gel, cleaning the cut surface, and replacing the stem in water, the leaves and the tip of the stem will begin to wilt. This plugs the vessels with gelatin. This demonstrates the slow penetration of water through the parenchyma tissues of the cortex and pith, which are not filled with gelatin.

The difference in the rate at which water will move in the vessels and in the parenchyma is dependent upon the method of transport. It flows through vessels as through hollow tubes, obeying the general laws of hydraulics. In the parenchyma cells, water is translocated osmotically, and its movement meets with considerable resistance.

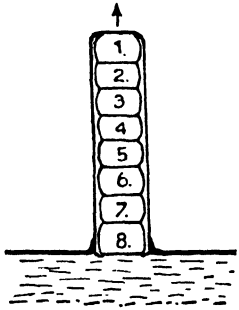


FIG. 103.—Diagram illustrating translocation of water in leaf parenchyma.

Imagine a vertical series of cells of which the lowest dips into water, while the other cells are above its surface (Fig. 103). In order to simplify the scheme, assume that only the upper cell evaporates water, while the other cells are protected against water loss by impermeable lateral walls. When all of the cells are saturated with water, there will be no movement of water. As soon as evaporation begins, the upper cell loses part of its water, the volume of the cell decreases, turgor pressure becomes less, and, as a consequence, suction tension develops (Art. 6). The upper cell now begins to draw water from the one beneath it, which up to that moment was saturated with water and, therefore, showed no suction tension. The loss of water creates a suction tension in the second cell, and it begins to draw water from the third, and so on, until the lower cell is reached. Then this cell begins to absorb water from the container, in which its lower part is immersed.

It must be remembered that the moving force that drives the water current from cell to cell is the difference in their suction tensions and not in the absolute magnitude of their osmotic pressures. Let the osmotic pressure of the first cell be 10 atmospheres, for instance; and of the second, 20 atmospheres. As long as they are saturated with water, they are in equilibrium with one another, as the surplus of pressure in the second cell is balanced by the greater tension on its walls. But as soon as suction tension rises in the upper cell, it begins to draw water

from the lower one, in spite of the fact that osmotic pressure is greater in the second cell.

Thus in order to make the water current, induced by evaporation in the upper cell, move through a series of cells, suction tension must be distributed in these cells in regularly decreasing order. In the upper cell, suction tension must be the greatest; in the following cell, it must be less; in the next, still less; etc. The least suction tension must be in the lowest cell, which absorbs water from the surrounding medium.

The difference in suction tension of two neighboring cells is determined by the resistance met by the water current in passing from one cell into the other. This resistance, in its turn, depends on and increases with the rate of the water flow. Ursprung was able to trace the increase of suction tension in the cells of the palisade parenchyma of the leaf, in proportion as these cells were farther from the large fibrovascular bundles supplying them with water. In the third cell from the bundle, he found a suction tension of 12.1 atmospheres; in the two-hundred-and-tenth cell, 32.6 atmospheres. In this case, in order to traverse 207 cells, a total difference in suction tension of 20.5 atmospheres was required, or about 0.1 atmosphere per cell. In other instances, somewhat different figures were obtained, but still they were close to this value.

The strong resistance met by water in moving from cell to cell shows that long distances cannot be covered in this way; hence, the necessity for more rapid means of translocation of water through dead, empty, elongated cells with numerous pores, the tracheids, or still better through hollow tubes with very thin septa, the tracheae, or vessels. Lower plants, such as the lichens and mosses, have no true vascular systems. Therefore, they are limited in size. The development of tracheids in ferns was an important step forward in the evolution of plants.

75. Translocation of Water in the Vessels. Forces Causing the Movement of Water. The Pulling Action of Transpiration.—The vessels and tracheids, being mere tubes filled with water, possess of themselves no source of energy that can cause the movement of water. On account of the very small dimensions of these elements, capillarity might be considered to be the only force operative in them. Confronted by the problem of the upward movement of water through the vessels, early investi-

gators, therefore, tried to explain this phenomenon by capillarity. The source of energy causing the capillary rise of fluids in narrow tubes is the force of adhesion between water and the vessel wall, shown by the concavity of the upper meniscus. But the plant vessels are completely filled with water. No meniscuses are found in them. Moreover, even if such meniscuses existed, with an average diameter of the vessels of 0.1 mm., water would be able to rise by capillarity only about 30 cm. In trees, the water has to be lifted many meters. Hence, the theory explaining the upward movement of water in the plant by capillarity was soon discarded.

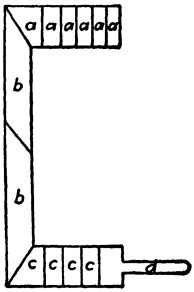


FIG. 104.—Diagram showing the arrangement of the terminal mechanisms of the water current: leaf and root parenchyma with vessels connecting them: (*a*) leaf parenchyma; (*b*) vessels; (*c*) root parenchyma; (*d*) root hair (*adapted from Walter*).

The water-conducting tissues being passive elements, the active movers of the water current are the living cells that adjoin the vascular system in its upper and lower end. At the base of the plant, this work is performed by the cells of the root parenchyma, which are in close contact with the vessels; while above, it is accomplished by the cells of the leaf parenchyma, which surround the tips of vascular bundles (Fig. 104; also in detail in Fig. 102). As a result of the activity of these cells, the water current moves through the dead tissue of the wood.

The work of the lower terminal mechanisms has already been discussed in Art. 65. It has been mentioned that the root-parenchyma cells are able to pump water into the vessels with a force of 2 to 3 atmospheres. Hence, with low transpiration the surplus of water may be forced out of a plant in the form of drops. The importance of this lower motive force, however, is not nearly so significant as is that of the upper terminal motor, the transpiring cells of the leaf parenchyma.

That transpiration is a sufficient source of energy to move the water supply of the plant may be seen from the well-known fact that portions of a plant, when cut off and placed in water, preserve under favorable conditions their freshness for a long time, using up a considerable amount of water. The mechanism of the transpiration pull is not so very complicated after all. It is

based on the fact already discussed that every cell not fully saturated with water shows a suction tension, which may reach several atmospheres. Hence, when brought into contact with water, it absorbs water with a proportional force. The parenchyma cells of a leaf, for example, will absorb water from vessels with a considerable force. This avidity for water will be the greater, the more the cell loses it, or the stronger the process of transpiration is. In leaves of trees, it reaches usually from 10 to 15 atmospheres. The upper terminal system thus represents a self-regulating mechanism that absorbs water in proportion to the rapidity with which it is spent.

The suction of water by the transpiring leaves can be readily measured. A leafy shoot may be conveniently used for this purpose. It is inserted into the upper end of a long tube filled with water, the lower end of which dips into mercury (Fig. 105). After a short time, it will be seen that as the water filling the tube is used up, it will be replaced by mercury, which may rise to a considerable height. The demonstration is brought to an end, not by the ceasing of transpiration, but by an accessory phenomenon. Air from the intercellular spaces of the shoot will begin to enter the tube, finally filling its whole upper end and breaking the connection between the vessels of the plant and the water.

This entry of the air into the tube interferes with the exact determination of suction tension of the leaves as measured by the rise of the mercury. Indirect measurements, based on the determination of suction tension in separate cells of a leaf, have shown, however, that a force of several atmospheres is attained. Thus, the upper terminal motive force shows a number of advantages over the lower one. It is able to develop a much greater force; it is self-regulating; and it works at the expense of radiant energy, which heats the leaf and increases transpiration. The lower mechanism works at the expense of nutritive substances supplied by the leaves. It is little wonder, therefore, that the upper equipment is more efficient than the lower and that most of the water used by the plant is supplied by its action. Only in early spring, when the leaves have not yet expanded, or in case of a very moist atmosphere, when transpiration falls to a minimum, is the principal role in raising water assumed by the root cells.

76. The Rise of Water in the Trunk of a Tree. Water Tension in the Vessels. The Theory of Cohesion.—The action of the upper and lower terminal mechanisms readily explains the rise of water through several centimeters and even through several meters in the stems of herbaceous plants, shrubs, and even small

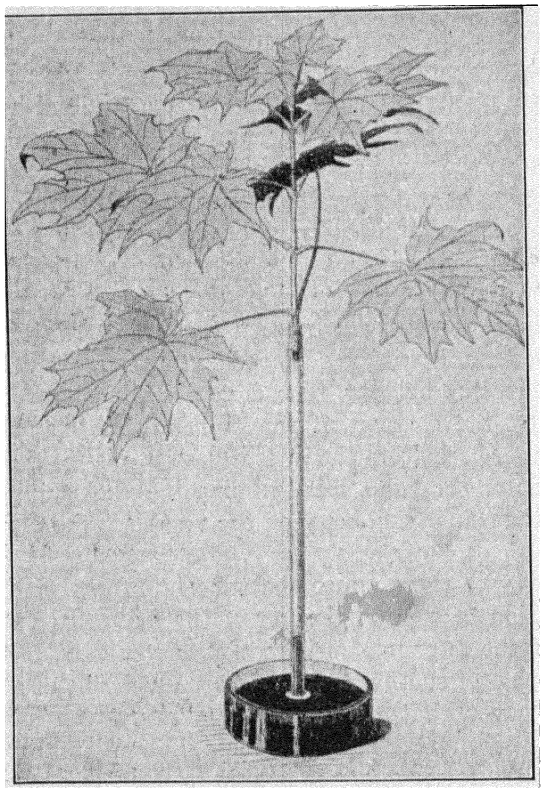


FIG. 105.—Transpiration pull shown by rise of mercury in the tube (*after Molisch*).

trees. In extending this explanation to the lifting of water in large trees to a height of 100 ft. or more above the soil surface, difficulties are met with that will now be discussed.

In the first place, root pressure is too feeble a force to drive water with sufficient rapidity to great heights. Only in the spring when root pressure is especially high and the loss of water from the undeveloped leaves is very low, may it be pumped with such force by the roots that the sap will exude from wounds of

the trunk and cut shoots. As soon as the leaves are completely unfolded, the force of transpiration will be so much greater than root pressure that cut roots will even absorb water. When larger roots are cut in summer, their surface does not exude water but absorbs it.

In the trunk of a tree, the suction tension of the leaves is transmitted downward to a considerable distance. It is known, however, that a suction pump is not able to lift water to a greater height than about 30 ft. At this height, the weight of the water column is equal to the pressure of 1 atmosphere. Pumping a perfect vacuum will not induce water to rise above this level, because the rise of water is due to the pressure of the atmosphere.

The cause of such a sharp difference between the lifting of water by a pump and the sucking of water in the vessels of the plant is explained by the fact that in plant vessels there are no air bubbles, which are always present on the surface of the walls of pumps. These air bubbles expand under tension, and the cohesion of the water column is broken.

The force of attraction between the water molecules is called "cohesion." It is the same force that holds an iron bar together, making it possible to lift it by one end. The force of cohesion in solid bodies may be determined in a comparatively simple manner because of the low mobility of their molecules and their constant external form. When the tensile strength of a bar of metal is tested, the strain at which it breaks is considered as being equal to the force of cohesion. Thus a steel bar, for instance, breaks at a strain of about 80 kg. per square millimeter, which approximately corresponds to the tension of 8,000 atmospheres.

Since liquids have molecules in a more mobile state, tests of the magnitude of their cohesive force are subject to greater difficulties and errors. The determination has to be conducted in a tube with two very closely fitting pistons. It has been proved by means of proper equipment that the cohesive force of water molecules and their adhesion to the surface of the pistons are very high. Only by applying to the pistons a pulling force of many atmospheres can the water layer between the pistons be ruptured. Still it is difficult to establish whether the water column itself has been ruptured or whether it has only been torn away from the surface of the piston or of the tube. The latter seems more probable, since on the surface of glass or metal there is always a

thin adsorbed layer of air, which prevents the perfect adhesion of water and probably induces premature rupture of the water column.

That the cohesive force of a liquid allows it to rise to a height exceeding that permitted by barometric pressure was proved by Askenasy's well-known experiment (1895). He took a long glass tube to whose upper end a funnel containing hardened plaster of Paris was sealed. The tube was filled with boiled water, and the plaster block filling the funnel was soaked with it to exclude gases. The lower end of the tube was dipped into mercury. Care was taken that no air bubbles were left anywhere (Fig. 106). The mercury in the tube began to rise after a short time, owing to the evaporation of water from the surface of the plaster-of-Paris block drawing upon the supply in the glass tube. If all the air was removed from the apparatus and the pores of the plaster block were sufficiently small to prevent the entry of air from outside, then the mercury rose finally to a height somewhat exceeding that caused by barometric pressure, as a result of the cohesion of the water molecules and their adhesion to those of mercury. This experiment clearly demonstrates the role played by cohesion and the effect of water evaporation from the surface of a moist porous body. The force of adhesion demonstrated by the concavity of the meniscuses in the capillaries of the plaster-of-Paris block is the active force in this case, the energy for lifting the water and mercury coming from heat energy of the environment. The greater the resistance to a pull, the more concave will be the water meniscuses and the stronger the tension on them. Transpiration pull evidently is based on this mechanism of capillary tension. The cell walls, from the surface of which evaporation takes place, are made up of cellulose in a colloidal state. The wall pores may be regarded as microcapillaries with their micromeniscuses of water. When the resistance to water flow is increased, these meniscuses are drawn into the tiny capillaries; they increase in concavity; and as a consequence, suction tension is augmented. Therefore, they draw water from the cell, inducing in it a suction tension of corresponding magni-

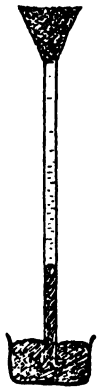


FIG. 106.—Suction produced by evaporation of water from plaster of Paris (*after Walter*).

tude and osmotic in nature. This pressure in its turn induces the movement of water by the cell from the vessels of the plant.

It is not possible to raise mercury very high in a glass tube, for the surface of the glass is always covered with a layer of adsorbed air. To approach the conditions found in a plant, Ursprung used, instead of a glass tube, the stem of a liana. He obtained a rise of mercury twice the barometric height, since the walls of the vessels in a plant stem are not merely moistened but saturated with water.

These experiments still do not give a true concept of the real magnitude of cohesive force that holds the water molecules

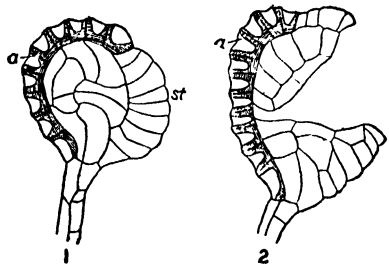


FIG. 107.

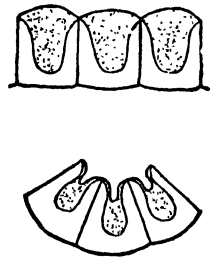


FIG. 108.

FIG. 107.—Sporangium of a fern; (1) with curved, (2) with straightened annulus.
FIG. 108.—Cells of the annulus of a fern sporangium; above, saturated with water; below, shrunken as a result of desiccation (*after Walter*).

together and prevents the rupture of the water column in the vessels of a plant. The cohesive force of water cannot be expressed in a few atmospheres. The simultaneous experiments of Renner and of Ursprung (1915) have shown that it may be as high as 300 to 350 atmospheres. These experiments were conducted on the annuli of the sporangia of ferns (Fig. 107). These annuli are made up of dead cells, whose inner lateral walls are thickened, while the outer walls are thin. In cross section, such a cell looks like a horseshoe, the ends of which are connected by a thin wire. When the sporangium matures, these cells, originally filled with water, dry up. The volume of water in them decreases, and the cells gradually shrink, drawing the thin walls inward, so that the ends of the thick walls approach each other (Fig. 108). Thus a highly strained spring is obtained, tending to rupture the water within the cells, or at least to tear it from the walls. The water column finally does rupture; the strained springs straighten at once; the whole annulus rapidly

contracts, scattering spores as from a catapult. The whole process may be readily observed under a microscope. The force necessary for such a compression of the cell walls in the annulus was determined by immersing them in highly concentrated solutions of various substances. It proved to be of the magnitude of 350 atmospheres.

This immense force of cohesion of the molecules is more than sufficient to raise water to the top of the highest tree, as the pressure of a water column 100 m. high is equal to but 10 atmospheres. Hence, the ascent of water in a tree may be represented in the following way. Near the top is the evaporating leaf parenchyma, in whose cells there develops a suction tension of many atmospheres. These cells draw water from the vessels of the vascular bundles, which permeate the whole leaf. Consequently, a pull on the water in these vessels is produced, which is transmitted to the vessels of the stem and roots. The water in the wood appears to be suspended to the cells of the leaf parenchyma. But in the root tips are also parenchyma cells, which, with a sufficient amount of water in the soil and with a slow utilization, support, as it were, the water threads suspended from the leaf cells. Thereby the strain on the leaf parenchyma is reduced, and the water threads are even pushed upward with considerable force. With lack of water in the soil and with simultaneous intensive transpiration, as, for instance, on hot summer days, the supply of water from the root cells cannot keep up with the loss of water from the leaves. In this case, the suction tension of the leaves is transmitted through the columns of water under high tension in the vessels, even as far as the root cells. This scheme has been worked out in detail by Dixon (1901). Since it attributes a conspicuous role in the ascent of water to the forces of cohesion, it is termed the "theory of cohesion."

The water columns filling the vessels tend to increase in length and to decrease in diameter, like a stretched rubber tube. This tendency would soon cause their breaking into separate drops, which is actually observed when water falls in a thin jet, if they were not contained within the walls of vessels to which they are closely attached by the force of cohesion. When water is under a very high strain in the vessels, the walls of the vessels will be drawn inward, and the diameter will decrease. This transverse

compression of the vessels is counteracted by the annular or spiral thickenings of the vessels, which keep the walls apart. Therefore on the whole, the compression is rather small. Nevertheless, it may be perceived under the microscope by observing the vessels of plants that have a sufficiently transparent stem—for instance, young plants of pumpkin or balsam, during rapid wilting, when the suction tension of the leaves attains its maximum, while the roots are unable to supply water from the dry soil.

The elastic compression of the walls of vessels during periods of increased transpiration is the cause of a phenomenon that has been known for a long time but has usually been misinterpreted. If on a hot day the stem of a rapidly transpiring plant is cut, the air will quickly enter the vessels. When placed in water, such a stem will soon wilt, as the air clogs the vessels, preventing water from rising. If the stem is cut under mercury, the latter will enter rapidly into the vessels and rise to a considerable height. In order to keep plants fresh, cutting must be done under water.

The reason for this phenomenon is that the vessels of the plant, being compressed with the force of several atmospheres, widen abruptly when the strained water threads are cut. Air, mercury, or water, depending on the medium used, will rush into the empty space. Yet for a long time this phenomenon was explained in a different way. It was supposed that besides water, the vessels contained highly rarefied air in the form of small bubbles. When a cut was made, this air contracted and drew after it mercury or water. The recent investigations by Renner and his coworkers have shown, however, that vessels that conduct water normally do not contain air but are filled with water throughout their length. Air will enter only as a result of a mechanical injury. If cutting is done carefully under mercury, the meniscus of the latter will join the water, no air bubbles being present in the vessels. The supposition of the presence of air bubbles in the vessels, forming the so-called "Jaminian chains," has prevented for a long time the acceptance of the cohesion theory, as these bubbles would interfere with the translocation of water and would, moreover, rupture water threads at such high tensions as are observed in the trunks of trees. It is known now that air is present only in the older wood, which has ceased to function for water conduction. The younger parts, through which water

moves, contain no air. Air that enters the vessels as a result of mechanical injury such as wounding, for instance, is not able to move farther than through the first septum. Such injuries will cause the exclusion of only small portions of the general system of water supply. The tracheid walls in many trees such as conifers are provided with bordered pits, which serve as check valves to prevent the entrance of air into the vessels.

The presence of bordered pits and other such structures in the tracheids and vessels, which necessarily offer a considerable resistance to the translocation of water in the wood, is nevertheless an indispensable protection to the regular functioning of the whole water-conducting system. The air, which under high tension may enter and clog the vessels, can be dissolved again when the tension is relieved; and the vessels that have temporarily suspended their functions may then once more begin to function in the conduction of water.

77. The Rate of Water Movement in Plants and the Resistance of Wood to the Ascent of Water. Participation of Intermediate Motive Forces in the Translocation of Water.—As wood consists of exceedingly narrow tubes, which moreover are provided with septa, it shows a strong resistance to the ascending water. Naturally, this resistance becomes an additional encumbrance to the cells of the leaf parenchyma, which carry the weight of the suspended water threads.

This additional resistance was formerly rated very high, considering the pressure necessary to force water through pieces of wood. The comparatively low rate at which the water moved in the plant, however, was not taken into consideration. Exact determinations by Ursprung have shown that with increase in height in the trunk, the suction tension of the parenchyma cells adjoining the vessels increases. But this increase constitutes only 0.3 to 0.4 atmosphere per meter in height. This is a small value indeed when compared with the increase in tension of water moving through the parenchyma. In this movement from cell to cell, the suction tension, as has been stated in Art. 74, increased by 0.1 atmosphere. If the average dimensions of these cells are assumed as 0.1 mm. since they are really one-third to one-half as large, it will be found that the translocation of water in the parenchyma for a distance of 1 m. would require the immense

force of 1,000 atmospheres. This shows the value of special conductive tissues for dry-land plants of large size. They increase the movement of water 2,500 to 3,000 times.

The actual translocation rate of the water through wood is not high. According to the calculations of Farmer, for deciduous trees it is, on an average, 20 cc. per hour per square centimeter of cross section of wood, and for conifers only 5 cc. If these values are compared with the rate at which water moves through ordinary city mains, which often reaches 100 cc. per square centimeter of cross section per second, or with the rate at which the blood is conveyed through the arteries, normally 40 to 50 cc. per second, it is found that water moves through ordinary water pipes several thousand times more rapidly than through a plant. With such slow translocation of water, the resistance to filtration cannot be very great. The suction tension developed by the cells of the leaf parenchyma seems to be quite sufficient not only to suspend the whole mass filling the wood, but also to draw it upward.

When the diameter of the vessels is increased and the number of septa decreased, the resistance of the wood to the translocation of water is considerably lowered. Such increased conductivity is found in lianas, the twining and creeping plants of tropical forests. The individual vessels in their stems may be 2 m. long and 1 mm. in diameter. These are vessels of exceptionally large size, however. In the majority of deciduous trees, the vessels usually do not exceed 10 cm. in length and 0.2 mm. in diameter. Such smaller dimensions, though reducing conductivity, give certain other advantages to the plant. The small diameter diminishes the danger of breaking of the water column, while the septa prevent the air, which for one reason or another may have entered, from spreading through the whole system.

Therefore it is seen that in the presence of a cohesive force, the work of the upper and the lower terminal mechanisms is quite sufficient to lift water to the top of a tree. The main containers of the water stream remain as passive as the iron pipes of a water system. Formerly, however, the cohesion theory was not elaborated sufficiently, and the resistance of the wood to filtration and the role of the air entering separate vessels were considerably overrated. For this reason, the idea of the insufficiency of the

terminal forces frequently presents itself, and the necessity was felt of looking for some intermediate mechanism situated in the trunk, which might supplement the terminal ones.

Most frequently the living cells of the wood were regarded as such an intermediate mechanism, especially the cells of the medullary rays and those of the wood parenchyma, which adjoin the vessels. It was supposed that these cells have the same function as the cells of the root parenchyma, of pumping water through them in one direction only. Lately, this view has been strongly supported by the Indian scientist Bose. This author tries to explain not only the translocation of water in the stem, but also the loss of water in transpiration, by the pulsation of the living cells. He contends that the water moves not through the wood but through the live cells near the cambial region. That water is in fact able to move only in wood layers that are still alive seems to support the idea of the participation of the living cells. In the dead inner layers of wood, the vessels are filled either with resinous substances, forming the so-called "heartwood" of the tree, or simply with air. They no longer participate in water conduction.

This theory of intermediate mechanisms has been refuted by a number of experiments. Votchall (1897) has proved by his comprehensive work that in a living tree trunk, placed horizontally, water moves from the morphologically lower end to the upper one at the same rate as from the upper to the lower. This clearly shows the absence of any valves in the wood that could prevent the downward movement of the water. But without such valves, the supposed intermediate mechanism would not be able to work. Moreover, Strasburger (1893) and other investigators have shown that when poisonous solutions, such as picric acid, are introduced through cut vessels, they will rise unchecked in the trunk to a height of several meters, or even to the upper leaves, though they certainly poison all living cells on their way. Likewise, the unchecked rise of water through portions of a herbaceous stem killed by heat, freezing, or toxins has been observed. After some time, however, such killed portions of a stem cease to conduct water, and their vessels become filled with air. The exact reason for this behavior has not been established as yet. Some investigators suppose that it results from changes occurring in the properties of the cell walls under

the influence of heat or of chemical substances. Others are inclined to think that the living cells of the parenchyma, without directly participating in the process of raising water, exert an indirect influence by absorbing the air that appears in the vessels. This question requires further study.

78. Translocation of Organic Substances in Plants and Their Storage.—The organic substances produced in the leaf by photosynthesis and those stored as food reserves in seeds and other storage organs must be translocated to the place of their consumption. One of the first prerequisites for their translocation, *viz.*, their hydrolysis into more mobile compounds, has already been discussed. The more complex transformations that fats and proteins undergo also have been noted. The substances most used by a plant and most easily translocated in its structure are the sugars and amino acids, especially asparagine.

The initial synthesis of all these compounds takes place in the leaves, and it is not difficult to show by simple experiments that this is followed by an uninterrupted movement of these substances from the leaves. It is possible to demonstrate this by Sach's dry-weight method, by which one may also study the accumulation of substance during the process of photosynthesis (Art. 38). If one-half of a leaf is cut off and dried in the evening, and the other half in the morning, it will be found that in the latter case the weight per unit leaf area will be considerably less. Thus, Sachs noted that a square decimeter of leaf area of the sunflower weighed 0.8 g. in the evening, but in the morning only 0.7 g. In this case, the leaf lost during the night 0.1 g. of organic substance per square decimeter, or about 0.2 g. per leaf, if it is assumed that the average sunflower leaf has an area of 2 sq. dm. From this, it is seen that early in the morning, the leaves of plants are considerably lower in organic substances than in the evening. This translocation from the leaves, of course, goes on not only during the night but also during the day, apparently with an almost equal rapidity. But during the day the outgo is overbalanced by the production of substance in photosynthesis. This may be shown if the increase in weight of the halves of the leaves remaining on the plant is compared with the increase in weight of leaves cut from the plant but placed under conditions of sufficient water supply, moist atmosphere, etc., so that synthesis will proceed uninterrupted. It will be

found that the leaves that have been cut off accumulate considerably more dry matter, sometimes twice as much, as those remaining on the plant. This difference indicates the quantity of substances that has passed from the leaf into the stem during the day.

In the autumn, when the time of leaf abscission approaches, besides this uninterrupted outflow of assimilates, gradually diminishing with the decrease in synthetic activity, the movement from the leaves of another type of compounds, such as organic phosphorus compounds that are more firmly bound within them, is observed. Evidently this is connected with the gradual aging of the cells and the breaking down of a part of their integral substances. Falling leaves as a rule have a considerably lower content of nitrogen, phosphorus, and potassium than those still active. Calcium salts, on the contrary, remain in fallen leaves.

A translocation of substances of similar nature takes place in annual plants, in which at the time of maturation of the seeds all other parts of the plant, leaves, stems, and roots, are gradually emptied of food substances and finally die. Water-culture experiments with corn, for example, have shown that after blooming, the plants may be transferred to distilled water and still will give normal development of seeds. It is apparent that all those compounds of nitrogen, phosphorus, sulphur, and other elements, which are stored in the seeds, are taken from the vegetative parts. At the end of development, even the carbon compounds are taken from the older parts in greater amounts than they are synthesized. It has been observed that the average dry weight of a wheat plant, taken from the field, does not increase between the time of flowering and the ripening of the grain notwithstanding the rapid increase in weight of the grain, which in the end contains almost one-third of the total dry matter. Hence, it is apparent that the weight of the remaining organs of the plant must have decreased by the same amount. In plants with a continuous type of development, such as the tomato and cucumber, organic substances are removed continuously from the lower senescent leaves and branches, which may continue through a long period of the life of the plant.

The substances that are photosynthesized and then removed from the leaves are distributed throughout the plant. Part of them are utilized by the growing regions, the young developing

stems and leaves and the tips of roots. Another fraction is directed into the ripening fruits and seeds, the underground storage organs, or the living tissues of the trunk of the tree, where it is stored as a food reserve. The storage processes are exactly the reverse of those that take place at germination; from the incoming sugars and amino acids, there are formed polysaccharides, mainly starch, fats, and reserve proteins. The chemistry of the synthetic processes is not nearly so well known as the chemistry of hydrolysis of these reserve substances. It should be noted that in all of these processes water is given off. Hence, in general, the maturation of seeds and the ripening of the wood in the fall in preparation for dormancy proceed more rapidly in dry weather, when water is being removed in larger quantity.

79. The Mechanism of Translocation of Organic Substances through the Plant. The Theory of Münch.—Organic substances elaborated in the leaves do not move in any definite direction but are always translocated to the points where they are either consumed or deposited as reserves. They may either rise in the stem toward the growing point and to the developing fruit or descend to the roots and underground storage organs. Their movement is thus connected with their consumption. The mechanism of this translocation, however, remained obscure until recently the German dendrologist Münch (1929) succeeded after many years of investigation in revealing this mechanism. It is based on the flow of the liquid contents of the sieve tubes and other conductive cells to the place of their utilization under the influence of the osmotic pressure developed in the cells elaborating organic substances. Accumulation of an excess of water in these cells is precluded by the pressing out of water into the elements of the wood. This creates the condition of a continuous flow of the solution in a definite direction.

In order to obtain a clear concept of the motive forces determining this flow of organic substances, let us return to the osmometers examined in Art. 4. Suppose the reservoir of the osmometer immersed in water, the osmometer itself being filled with a solution. As has been seen, such an osmometer will absorb water so long as the weight of the water column in the tube does not exceed the greatest osmotic pressure that the solution is able to develop. The tube at the time of develop-

ment of the maximum osmotic pressure contains not pure water but the solution filling the osmometer. If additional liquid is poured into the tube, the level in the latter will nevertheless very soon return to its previous height. This is explained by the fact that the excess of hydrostatic pressure, as a result of the increase in height of the column in the tube not being counterbalanced by the osmotic pressure, will force a part of the water outward, through the walls of the osmometer.

Now let us suppose two similar osmometers *A* and *B* (Fig. 109) connected by means of a glass tube, through which the contents may circulate. Osmometer *A* is filled with a concentrated solution; osmometer *B*, with a weaker solution. For the purpose of demonstration, suppose the concentrated solution is colored with some dye. If both osmometers are immersed in one vessel with water *W*, it will be observed that their contents immediately begin to move. Osmometer *A* will begin to absorb water; a hydrostatic pressure will arise inside the system that will be higher than the osmotic pressure in the osmometer *B*; and as a result, water will be forced out of the latter into the surrounding vessel. The solution from osmometer *A* will then flow through the connecting tube *V* into osmometer *B*, replacing the water that is being pressed out of it. This process will continue so long as the concentrations in *A* and *B* are not equal. When they are the same in both vessels, the osmotic pressures will also become equal, and the whole system will be in equilibrium.

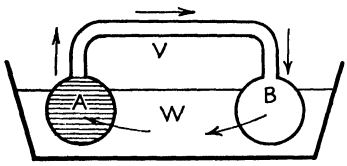


FIG. 109.—Diagram showing the movement of organic substances in plants. *A* and *B* are permeable membranes (osmometers) submerged in water and connected by a glass tube. The concentration in *A* is higher than in *B*. *V*, vapor; *W*, liquid.

The same result of the leveling of the concentrations in the reservoirs *A* and *B* may be obtained by means of diffusion of the dissolved substance, with the following essential difference, however. While with a flow, caused by pressure, equilibrium is attained in several hours, in the case of equalization by means of diffusion—for instance, if the reservoirs were made of glass—it would take many years to reach equilibrium. According to Münch's calculations, with the difference of the osmotic pressure in the two reservoirs being only 1 atmosphere, the velocity of

pressure flow exceeds that of diffusion 10 million times for a distance of only 1 mm.

From this physical scheme, let us pass to the plant. Osmometer *A* with the concentrated solution is represented by the leaf cell, which under the influence of radiant energy continually elaborates osmotically active substances, chiefly sugars. The connecting tubes, along which the solution of sugar and of other organic substance moves under the influence of pressure, are represented by the sieve tubes, the principal conductive elements of the phloem. Osmometer *B* with the low osmotic pressure, which squeezes out the water penetrating from the connecting tube, is represented by the cells utilizing organic substances, in which a low concentration is maintained either owing to growth and respiration, as in the growing meristematic cells, or as a result of the transformation of soluble substances into insoluble ones, for instance, the transformation of sugar into starch, as in cells of storage tissues of seeds and underground storage organs. The reservoir that receives the excess of water is represented by the dead water-conductive cells, the vessels of the wood, which always accompany the phloem and serve not only for water supply but likewise for the diversion of excessive water and thus provide conditions for a continuous flow of the nutritive solution. The tension usually prevailing in the vessels (see Art. 76) contributes to a considerable degree to the exudation of the excess of water from the developing cells.

In the living plant, there exists a very essential distinction as compared with the physical model examined above: *viz.*, instead of the direct communication between the two osmometers through a glass tube, in the case of the plant there is a series of cells separated from one another by septa and, what is still more important, by impermeable layers of protoplasm. The sieve tubes likewise consist of a series of cells separated from one another by sieve septa. This distinction does not contradict but only somewhat complicates the scheme presented above. In spite of the septa, the sieve tubes possess one continuous cavity; for the perforations in the sieve septa are so large that both the protoplasm of the separate cells and the liquid cell sap represent one continuous mass. Although the sieve septa increase somewhat the resistance to the flow of liquid, nevertheless they do not present a serious obstacle.

The scheme is more complicated with the passage of the solution of organic substances either from the assimilating cells into the sieve tubes or from the sieve tubes into the consuming cells, and in general with the transfer from one living cell to another. As has already been seen in Art. 5, living cells are almost completely impermeable to most dissolved substances, this impermeability being the cause of the high osmotic pressure prevailing in them. Sugars especially penetrate with difficulty into the cells. Nevertheless, sugars move very easily through the plant, and according to Münch's analysis they form the chief part of the sieve-tube contents. Münch finds the solution of this difficulty in the plasmodesmen, the fine plasmatic communicating threads that pass through pores in the walls of the cells connecting all the protoplasts of the plant into one complete whole, or symplast. It is through these plasmodesmen that filtration of the solutions takes place from cell to cell under the influence of pressure. The impermeability of the surface layer of protoplasm surrounding these fine threads hinders a disordered diffusion of organic substances, thus directing their flow toward the sieve tubes and finally to the cells where they are utilized.

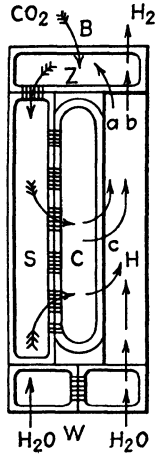


FIG. 110.—*B*, leaf parenchyma cell forming sugar, *Z*, from CO_2 and H_2O; *S*, sieve tube of phloem; *C*, cambial cell; *H*, xylem vessel; *W*, root cells. The straight arrows indicate the flow of water, the curved arrows indicate flow of sugar and other organic substances. The double outlines in cells *B*, *S*, *C*, and *W* indicate protoplasm (after *Münch*).

The main consumers of the organic substances in the trunk of the tree are the cells of the cambium, which separate the phloem from the xylem and continually form new tissues. According to Münch, the translocation of the organic substances in the tree trunk may be illustrated by the adjacent scheme (Fig. 110). The parenchyma cells of the leaves *B*, absorbing carbon dioxide from the atmosphere and water from the vessels of the wood, elaborate sugars *Z*. The increase of concentration creates osmotic pressure, which forces the sugar solution through the plasmodesmen into the sieve tube *S* and from there again through the plasmodesmen into the cambial cell *C*. The cambial cell consumes sugar in the process of growth; its osmotic concentration falls; and the excess of water is filtered back into

the vessel, as shown at the upper part of the scheme. The same sugar solution in the sieve tubes absorbs water from the root cells *W* and transfers it through the cambial cells, which retain the organic substances, into the vessels of the root and stem, thus creating root pressure, as shown at the lower part of the scheme.

Evaporation of water from the cells of the leaf parenchyma creates a transpiration stream, represented by simple arrows in the right half of the scheme, causing a tension of water in the vessels and thus contributing to the withdrawal of its excess from the cells of the cambium.

Translocation of substances in developing fruit, where there is little if any assimilation and transpiration, may be illustrated by the following simpler scheme (Fig. 111). The parenchyma cells *P* of the leaf and stem, creating sugar or elaborating it from less soluble reserve substances stored previously, attract water from the wood *H*. This creates an osmotic pressure, and the flow of sugar is transferred through the plasmodesmen to the sieve tubes and thence to the cells of the fruit *F*, where the monosaccharides are partly utilized in growth and partly stored in the form of polysaccharides or disaccharides. The concentration of the solution falls, and the water that is no longer necessary is removed along the xylem elements. Generally speaking, in the fruit, which evaporates very little water, the xylem elements serve chiefly for the outflow and not for the inflow.

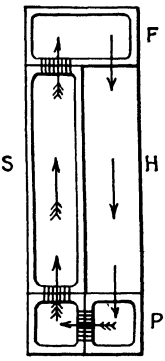


FIG. 111.—Scheme of sap movements in maturing fruits. *F*, fruit cells; *S*, sieve tube of phloem; *H*, xylem vessel; *P*, parenchyma cells of leaf and stem (*after Münch*).

Münch's theory, based on a series of ingenious considerations, exact calculations, and very careful experiments, has given a very successful solution to the question of the translocation of organic substances in the plant, which has held the interest of botanists from the time of the founder of plant anatomy, Malpighi (1675). Together with the theory of cohesion elaborated by Dixon (1901), with which it is very closely connected, Münch's scheme gives a sufficiently complete picture of the circulation of sap in the plant, somewhat analogous to the picture of circulation of the blood in animals. As yet, however, it cannot be acknowledged as completely proved, and its weakest point is the assumption that organic substances move through plasmodesmen.

Thus, at present, it must be considered only a very ingenious hypothesis, requiring further elaboration and verification.

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CHAPTER X

RESISTANCE OF PLANTS TO UNFAVORABLE ENVIRONMENTAL CONDITIONS

80. Effect of Low Temperatures on the Plant. Freezing of Plant Tissues and Processes Accompanying It.—It is only in tropical climates that plants remain all of the time under temperature conditions favorable for their life activity and can develop unhindered the year round. In temperate and even in subtropical climates, they have to endure the influence of temperatures below freezing, when their sap freezes and the plants that are not sufficiently resistant to the changes occurring in their tissues with freezing are injured or killed. The existence of plants in temperate climates is closely connected with their frost resistance. Some of them possess so short a vegetative period as to be able to accomplish their entire life cycle in the frostless period of the year. Such are the annuals, which possess a short vegetative period and begin their development after the termination of the spring frosts and complete it before the beginning of the first autumn frosts. Most of the plants of temperate climates that live through the winter in the vegetative condition have the ability of hardening sufficiently to endure the low temperatures of winter or have parts that vegetate underground and thus escape the low air temperatures. Among crop plants, the winter cereals and grasses, as well as several other groups that are of great agricultural importance, overwinter under the snow in the vegetative phase of their development.

Ability to survive the winter may be dependent upon several different growth habits of plants. Annual plants overwinter in the form of dry ripe seeds that will not freeze at low temperature. Most of the perennials lose their aerial organs and overwinter in the form of bulbs, tubers, and rhizomes well protected from the frost by a layer of soil and snow. But winter crops with parts above ground and woody plants must inevitably endure the influence of frosts; their tissues sometimes freeze to the

ground level or below it. Their ability to overwinter then must depend upon their hardening capacity.

An explanation of frost resistance is to be found in studies of the phenomena that take place in the cells and tissues of plants during freezing. Plants killed by frost look as if they had been scalded. They lose their turgidity, and their leaves rapidly turn brown and become dry. When such fleshy tissues as the potato

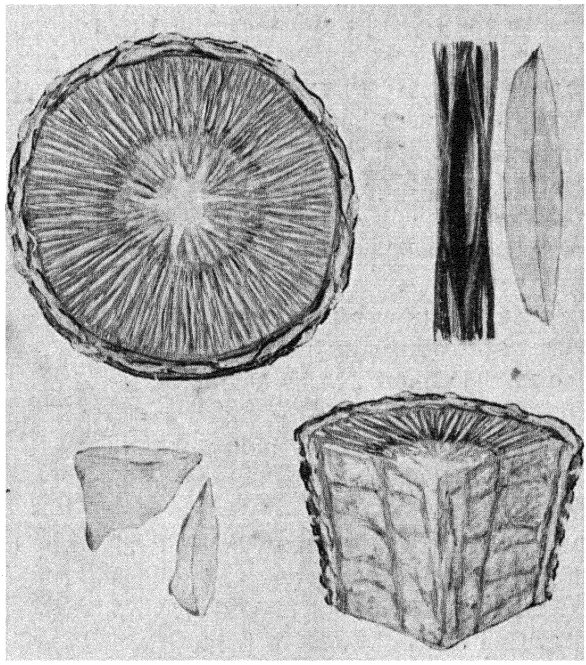


FIG. 112.—Ice masses in frozen carrot. Note arrangement of ice and breaking apart of tissues (*after Harvey*).

or the beet thaw out after having been frozen, water flows out of them as easily as from a sponge.

For a long time, this ready loss of sap by frost-killed organs was explained as being due to the fact that the freezing water, in expanding, ruptures the cell walls. This supposition, however, proved false. Microscopical observations have shown that water contained in the cell walls is frozen first and that ice is formed not within the cell but in the intercellular spaces (Fig. 112). The cell walls in frost-killed plants remain uninjured.

The cause of the death of these plants must be sought not in the rupture of the cell walls but in the changes of the protoplasmic membrane, primarily its coagulation. This coagulation is the result of the formation of ice in the intercellular spaces and the withdrawal of water from the cells. The cell sap becomes increasingly concentrated, while the protoplasm is gradually deprived of water. Besides, the protoplasm is exposed to the pressure of the growing ice crystals. As a result, an irreversible coagulation of the colloidal substances of the protoplasm takes place. After thawing, it is dead and has lost its permeability.

Hence, frost killing must be regarded not as the direct influence of cold on the protoplasm but as an indirect effect, the desiccation of the protoplasm due to the freezing out of water. One of the most direct proofs of this statement is the fact that in the condition of undercooling, without ice formation, the plant is able to endure such low temperatures as would kill it if freezing had occurred.

The increase in concentration of the cell sap occurring during freezing likewise exerts a harmful effect upon the protoplasm. The action of the anions and cations upon the protoplasm and especially of the hydrogen ion contained in the cell sap is intensified with freezing. The total acidity of the cell sap increases, and this may also contribute to a coagulation of protein substances of the plasma.

81. The Reasons for Different Frost Resistance of Plants. Hardening to Low Temperatures. Methods of Determination of Frost Resistance.—Not all plants succumb with equal ease to the formation of ice. Some of them, the most sensitive, are killed in the very beginning of this process. Others, such as the cabbage or the onion, are able to stand moderate freezing. Those in the third group may freeze to a hard condition and thaw out afterward without losing their vitality. Many plants of the polar regions have this capacity. Some winter cereals are able to stand frosts of -15 to -20°C. even in snowless winters. Still more cold resistant are the dormant buds of deciduous trees and the needles of conifers.

The reason for this hardiness was sought first in the fact that because of the small amount of water in these dormant organs, especially the buds and needles, no ice could form. This supposition, however, has proved erroneous. Observations have

shown that even these organs freeze at −15 to −20°C. With still lower temperatures, they become as brittle as glass. Moreover, all such external adaptations as the layer of cork covering the branches and the bud scales, which, for a long time, were regarded as protections against cold, in reality are unable to check heat loss for any considerable length of time. The role of these structures is a different one: *viz.*, they protect against desiccation during the winter, which threatens the aerial parts of the plant as a result of the decreased water supply from the frozen soil. Frost resistance is not a capacity of dormant plants to protect themselves against the formation of ice but a property of being very little or not at all affected by its formation.

This lack of susceptibility is attained as a result of chemical changes in cells. It has been noticed for a long time that during the cold season the leaves of certain plants, though wintering under snow, show no traces of starch. Considerable amounts of sugar are accumulated instead. Experiments have shown that sugar has the property of preventing the coagulation of protein substances at low temperatures. It therefore may be called a protective substance. This protective influence of sugar may be readily demonstrated by means of the following simple experiment: Take three test tubes containing the sap squeezed from a plant. This sap always contains a certain amount of protein. One of these tubes should be left as a check; the two others should be frozen, after a little sugar has been added to one of them. Upon thawing the contents of both tubes, it will be seen that in the tube that received sugar the proteins are in the same colloidal condition as in the control. The tube frozen without the addition of sugar will show a flaky precipitation of the coagulated protein (Fig. 113).

It is of interest to note that the sugar content in dormant plants is not constant. It increases with lower and diminishes with higher temperatures. The frost resistance of plants varies accordingly. It was noticed, long ago, that winter cereals when grown in warm greenhouses are easily killed by freezing, while those grown in the open at low temperatures show a high degree of hardiness. Even a prolonged thaw decreases perceptibly the cold resistance of plants. On the other hand, if plants grown in a warm place are kept for several weeks at a temperature but slightly above freezing, they can be hardened to withstand a mild

frost. Such hardening is always accompanied by an increase of the sugar content in the cells of the plant. A similar accumulation of sugar may be observed in potatoes stored at a temperature about 0°C.; the increase of sugars is readily perceptible to the taste. This unpleasant flavor is easily removed by keeping such sweetish potatoes for some time in a warm room.

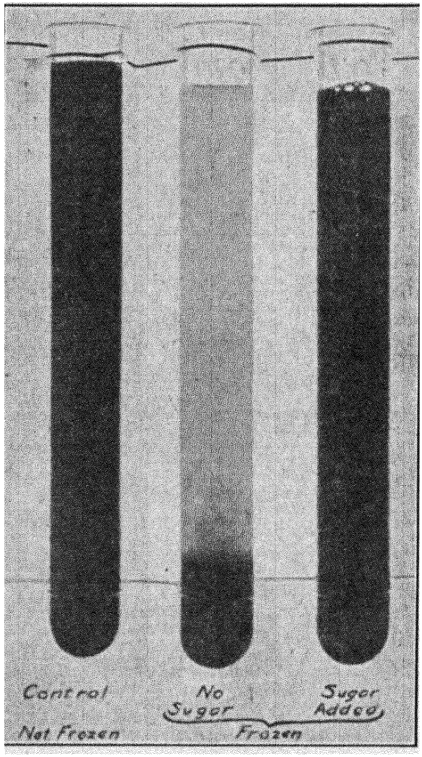


FIG. 113.—Protective influence of sugar in freezing expressed plant juice (*after Newton*).

Hardening, however, is not entirely due to the accumulation of sugar; the protein substances of the protoplasm also undergo changes, as a result of which they obtain the capacity of being coagulated irreversibly only at temperatures lower than before hardening. Especially marked seasonal variations in hardiness may be observed in trees. The needles of conifers, for instance, are able to withstand winter temperatures of −30 to −40°C., while in summer they are killed at −8°C.

These changes consist chiefly in the increase of the water-retaining power of the colloids. Owing to this property, during freezing they give up with great difficulty the water contained in them; and even at very low temperatures, a considerable amount of unfrozen firmly bound water remains in them. Besides, partial hydrolysis transforms the protein substances of the plasma from more complicated but unstable forms into simpler compounds that are not so easily dehydrated during freezing. In this process, a part of the amino acids is split off and accumulates in the cells.

Increase in endurance of low temperature is produced not only by the accumulation of protective substances and by changes of the colloids of the protoplasm but also by a general decrease in the amount of free water that may freeze. Dry seeds, containing only about 10 to 12 per cent of hygroscopic water, are absolutely unsusceptible to frost and can endure without injury the temperature of liquid air or even of liquid hydrogen. Soaked seeds are killed at 8 to 10°C., while seeds that have begun to germinate and have absorbed water abundantly are still more sensitive to cold. Likewise, wood of branches that have terminated growth, and are ripe and low in water content, is much more resistant to frost than the wood of growing shoots. In such previously dehydrated tissues, only insignificant quantities of ice are formed even during severe frosts, and this is why they are less susceptible to cold.

In the hardening of winter cereals, light plays an important role. With the approach of winter, these plants are quite young, tillering sometimes not having yet begun, and thus they do not have at their disposal a large reserve of carbohydrates. But the sugars formed by photosynthesis in winter contribute considerably to an increase in their frost resistance (Tumanov, 1930). Therefore, in regions where there are many clear sunny days in autumn, winter cereals are more hardy than in regions where cloudy days prevail. Likewise, in years with a clear dry autumn, winter crops are better hardened than in years with a wet and dark autumn.

In addition to the factors that increase frost resistance, there are also others that decrease it, as for instance the abundance of free water or the acidity of the cells. Different tissues, and even certain cells of the same tissue, may show various degrees

of frost resistance. In the seedlings of cereals, for instance, the most susceptible part is the plumule, and the hardiest are the root tips and their central cylinders, while cortical parenchyma of the root is comparatively little frost resistant. After moderate freezing, the root tips, therefore, continue to grow for some time, while the young leaves and the external portions of the roots are killed.

Frost resistance is likewise decreased as a result of alternate freezing and thawing, which greatly weaken the protoplasm because of the continuous change from a state of dehydration to that of saturation.

During autumn and winter, the frost resistance of winter plants changes continually. When sown in early autumn, during fairly warm weather, winter crops are not very frost resistant in the beginning of their development, and freezing without a cool period previous to it may be very injurious to them. But with a gradual decrease in temperature, they become more hardened. Clear sunny days alternating with moderately frosty nights are most favorable in this respect. Usually up to the time of snowfall, the winter crops are in their most hardened state and are capable of enduring the relatively severe frosts of the first winter months. The snow cover, which smooths considerably the fluctuations in temperature at this time of year, likewise exerts a considerable protective influence.

But a very prolonged period under snow, especially if the layer is thick and prevents the passage of the sun's rays, leads to a gradual decrease of frost resistance. As a result of continuous respiration, the plants gradually lose their carbohydrate reserve and cannot restore it because of insufficiency or even complete absence of light for photosynthesis. This is accompanied, besides, by other internal changes, which also decrease frost resistance, such as vernalization, which will be discussed in detail in Chap. XII. Toward spring, the plants are very weakened and may succumb easily to the influence of a mild frost, which they could endure without harm at the beginning or in the middle of the winter.

Not all plants can be hardened. Plants of southern latitudes, *e.g.*, cotton, rice, as well as cucurbits, after a prolonged exposure at temperatures just above 0°C. do not increase their frost resistance but on the contrary may be injured or killed. Rice and

cotton are especially sensitive. A temperature of 0 to +5°C. for 24 hr. may be lethal to them. But death of such plants from low temperatures differs in character from death following freezing. It occurs not all of a sudden but gradually and frequently is observed only after the plants are returned to a warmer temperature. The actual causes of death in such cases are not known. Death is usually preceded by the appearance of brown spots, by the destruction of chlorophyll, by a loss of turgor, and by abscission of the leaves. This general behavior leads to the supposition that there is a disturbance in the regular changes of the biochemical processes in the plants and that an accumulation of poisonous products of metabolism takes place, which further causes the death of the whole organism.

Various winter plants possess a widely differing frost resistance. Thus varieties of wheat originating in Eastern Russia are very frost resistant, while varieties from Southwestern Russia and Western Europe are very susceptible to frost. Still more susceptible are varieties originating from the Transcaucasus and Central Asia, where they are grown in a mild and almost frostless winter.

But the origin of the variety alone does not give a full knowledge of its resistance. For a systematic selection of winter varieties, it is quite indispensable to have more convenient and reliable methods of determining their frost resistance. Not so very long ago, the only method for this was the testing of varieties under field conditions during several winters, keeping an account of the number of plants that had overwintered in each plot. But the field method requires many years of testing; for in mild winters all the varieties overwinter well, and one has to wait for a more severe winter. Besides, the results obtained are not reliable; for death during the winter may be the result of injuries not only from low temperatures but likewise from fungi or insects.

That is why much attention has recently been given to the elaboration of rapid laboratory methods for the determination of frost resistance. In the first place, there may be mentioned the so-called "direct freezing method," or "artificial winter test" introduced by Harvey and widely used for testing the hardening capacity of plants in special refrigerators, which are cooled to the required temperature by means of refrigerating machines. The varieties intended for testing are sown in boxes filled with

soil and are hardened for 5 to 10 days previous to the testing, for in a nonhardened state the differences between the more and the less frost-resistant varieties are quite insignificant. After hardening, the boxes are placed for 1 or 2 days in the refrigerators at a definite temperature; and after gradual and careful thawing, the degree of injury by freezing is determined, the greater injury corresponding to a lesser frost resistance.

The method of direct freezing requires complicated and expensive refrigeration installations and hence is accessible only to large institutions. The so-called "indirect methods" of determining frost resistance are therefore frequently applied. They are based on the fact that in genetically closely allied plants, *e.g.*, in different varieties of wheat or rye, the degree of frost resistance varies more or less proportionally to their chemical composition, for instance, to their sugar content. The latter may be readily determined either directly by chemical analyses or more quickly by means of a refractometer, which is widely applied for this purpose in the sugar industry. Another group of indirect methods is based on the determination of the amount of water bound by colloids. All these methods, like the method of direct freezing, can be used only with such plants as have been sufficiently hardened.

Indirect methods are more rapid, more convenient, and less expensive than the method of direct freezing, but they give less reliable results; for they allow one to determine only one of the items, the sum of which determines the degree of frost resistance. For example, with one and the same sugar content, the frost resistance of the varieties compared may be different because of variation in the amount of bound water. Hence, indirect methods can serve only for a very approximate preliminary estimate of the hardening capacity of varieties.

82. Other Harmful Effects during Wintering: Exhaustion, Drowning, Heaving, Winter Desiccation. Physiological Basis of Selection for Winter Resistance.—The direct influence of freezing upon the cells is not the only danger threatening plants during winter. They often perish from other unfavorable conditions. Death of plants from exhaustion is usually observed in mild winters with a deep snow cover, especially if the snow falls on thawing soil. For a long time, this was explained by the suggestion that the plants remaining at a relatively high tem-

perature, about 0°C. or even somewhat higher, respire intensely, utilizing all the oxygen at their disposal. The thick snow cover inhibits the access of fresh air, and the plants suffocate. However, recent investigations (Tumanov, 1932) have shown that even under a thick layer of snow the air in direct contact with the plants contains sufficient oxygen and that the cause of their death is not suffocation but exhaustion of reserves. Remaining at a temperature about 0°C., when respiration is sufficiently intense, and being at the same time in almost complete darkness, which precludes the possibility of photosynthesis, the plants gradually exhaust all their reserves of nutritive substances and finally die of starvation.

Resistance to exhaustion is conditioned by peculiarities other than resistance to freezing, and thus hardy varieties are not always capable of enduring prolonged periods under a thick snow cover. A low respiratory intensity at low temperatures plays an important role in resistance to fungi; but as in the case of hardening, the accumulation of large carbohydrate reserves is likewise of great significance.

Another danger, which must not be confused with exhaustion, is injury from an excess of water. It is frequently observed in spring during prolonged periods of thawing, when melted water accumulates on the surface of the frozen soil and floods the plants. In this case, the death of the plants is a result of suffocation from oxygen deficiency. In the snow water surrounding the plants, alcohol, the chief product of anaerobic respiration, may be detected. If with the return of cold weather this melted water freezes again, forming an ice crust entirely covering the plants, the latter, being previously weakened by suffocation, are almost sure to die. Ice crust is one of the frequent causes of death of winter cereals in large areas, especially in the southern part of Western Russia.

If the melted water has been absorbed by the soil, a new danger arises with the subsequent freezing of the soil, *viz.*, heaving, which breaks the roots of the plants. The freezing of the soil water begins very often not from the surface but at some depth on the boundary of the soil layers that have not thawed. An ice layer is formed that thickens gradually and lifts the upper soil layers together with the plants, thus pulling them out of the soil. The roots that in autumn have penetrated to a con-

siderable depth are broken off. In spring, when the ice layer thaws and the soil that had been lifted by it settles down again, the plants with the ruptured roots remain lying on the surface and are easily killed by desiccation, if the spring is not moist enough to allow them to form new roots. Their recovery may be promoted by well-timed rolling of the field.

Plants whose overwintering organs are situated largely above the soil, chiefly shrubs and trees, are subject to an additional danger in the winter, *viz.*, excessive desiccation. Though the evaporation of water is insignificant at temperatures below zero, nevertheless the water balance of shrubs and trees in winter is unfavorable for them owing to a complete cessation of water absorption from the frozen soil. With strong permanent winds or on clear sunny days, especially toward the end of the winter when the sun's heat is considerable, the plants may lose too much water and die as a result of drying out. Autumnal drought is a major cause of the abscission of leaves of deciduous trees and the formation of a thick layer of cork on branches of trees, which are not found in grass plants with annual stems. Leaves of conifers and other evergreen plants develop a thick cuticle, which lends them the characteristic rigidity and protects them from water losses.

The snow cover prevents the desiccation of winter cereals, and it is only in the absence of snow during a prolonged period in autumn that wilting may be observed. But this may be more profitable than harmful to the plant; for it increases considerably the frost resistance, which is especially important in the absence of a snow cover. But an excessively early and severe drought in autumn checks normal tillering and rooting and inhibits the accumulation of protective reserve carbohydrates, thus proving harmful for winter crops.

The causes of winter injury and winterkilling of plants are various and are not determined by the action of freezing alone. Hence, the concept of winter resistance, or the capacity to withstand diverse harmful effects that occur during winter, does not coincide with the more narrow concept of resistance to freezing.

When selecting winter-resistant plants, it is not sufficient to determine frost resistance alone by means of one of the methods discussed. It is necessary to subject the plants to the complex

influences of all the unfavorable factors. This may be best achieved under the conditions of field experiments.

In various climatic regions, different factors may be of major importance. It is quite natural, therefore, that varieties that are winter resistant in some regions may be considerably less resistant in others. For instance, the extremely frost-resistant varieties of the Volga region, where the killing of winter cereals is conditioned almost exclusively by winter temperatures with insufficient snow cover, when cultivated in West-central or Northwestern Russia yield the first place to the local winter-resistant varieties, which better withstand rotting, a phenomenon common in these regions. In systematic selection for frost resistance, it first of all must be ascertained which of the harmful winter factors is the most important in the given region. Great attention should be directed to the study of resistance in relation to these leading factors. The influence of these numerous factors being very diverse and even to a certain degree contradictory, we cannot expect to select such a variety as would have winter resistance in all regions.

83. Effect of High Temperatures on Plants. Heat Resistance and Its Physicochemical Basis.—In examining the effect of temperature on the various physiological functions, it has repeatedly been mentioned that beyond the optimum temperature, when a process reaches its highest rate, there follows a decrease up to the maximum temperature, when it ceases entirely. This sharp decrease in the rate of the separate physiological functions at temperatures above the optimum is explained by the fact that beginning from certain temperatures pathological processes begin to prevail in the plant, which suppress the normal physiological functions and finally cause the death of cells.

The action of superoptimal temperatures as it is usually specified is often too simple. It is assumed that the whole phenomenon can be explained by the coagulation of the protein substances of the protoplasm at high temperatures. Actually, the phenomenon is much more complicated; for death usually begins at temperatures slightly above 40°, which are far below the coagulating point of proteins. Probably, as in the case of thermophilous plants, at temperatures close to zero, death is preceded by a disturbance in the coordination of the biochemical processes taking place in the cell and by an accumulation of

poisonous substances of the type of toxins, which poison the protoplasm. At higher temperatures, about 50°C. and above, this toxic effect is aggravated by the coagulation of the protoplasm, and this leads to a very rapid death of the cells.

Various plants differ in their degree of resistance to high temperatures. Blue-green algae and bacteria living in hot mineral springs at temperatures about 70°C., at which all other organisms quickly die, are the most heat resistant. Of the higher plants, succulents of the type of cacti and different representatives of the family of *Crassulaceae* endure without injury heating by the sun's rays up to a temperature of 50 to 60°C. Plants of a xerophytic type are more resistant than mesophytes. The most heat resistant of the crop plants are the thermophilous varieties from the southern latitudes, such as sorghum, rice, cotton, and the castor-oil plant.

In estimating the degree of heat resistance of plants in natural environments, it must be kept in mind that the temperature of plant tissues may not coincide with that of the surrounding air. In spite of the fact that the leaves absorb radiant energy, their temperature in the hot noon hours may be considerably below the air temperature, for most of this energy is consumed in the evaporation of water. Unimpeded transpiration protects the leaves from excessive heating. Hence, dry heat is more easily endured not only by man but also by plants. Plants transpiring intensely are less subject to overheating by the sun's rays. This explains the high transpiration intensity of many desert plants. Wilting plants with closed stomata suffer more readily from overheating than those that are sufficiently provided with water.

Nowhere on the earth's surface, with the exception of the volcanoes, are there found places where plants could not develop on account of high temperatures alone. The barrenness of deserts is explained not by excessive heat but by a deficiency of water. Sometimes, with hot dry winds the temperature may rise beyond the limits that can be endured by plants, and in such cases, the crop plants may be severely injured in a short time. The hot winds being dry as well, it is sometimes very difficult to distinguish the direct effect of a high temperature upon the protoplasm from its indirect influence as a desiccating agent According to Zalensky's observations, in hot dry air the stomata

of plants open widely under the influence of high temperature. This pathological phenomenon takes place even with deficiency of water in the leaves and leads to a rapid desiccation and death of the plants.

Although not surpassing the limits endurable by plants, temperatures from 35 to 40°C. may be very harmful if their action is prolonged. The disadvantageous change in the relation between respiration and photosynthesis is of major importance in this case; photosynthesis possesses a relatively low optimum as compared with respiration. At high temperatures, the expenditure of organic substance in the process of respiration exceeds its synthesis, and the plant is gradually depleted of its carbohydrates and then begins to starve. This is especially the case with plants in moderate climates, such as wheat, flax, and many truck crops, which at very high temperatures begin to elongate, tiller little, and decrease their yield. Besides, this general weakening makes them more susceptible to fungous attacks. The harmful effect of excessively high temperatures is one of the chief causes of decreased yield of early spring cereals when they are sown too late.

In different growth stages, plants show various degrees of susceptibility to the effect of high temperatures. Usually, excessive heat is most injurious in the early stages of growth. Conversely, during ripening of the fruit, high temperatures may be beneficial. For some plants, high temperatures are especially injurious during flowering and cause sterility and abscission of the blossoms or young fruit.

84. Effect of Water Deficiency on the Plant. Soil and Atmospheric Drought.—One of the adversities of the plant most often met with is the deficiency of water, which in its extreme manifestation is designated as "drought." Drought, first of all, disturbs the water balance of plants. This sooner or later disarranges all the other physiological functions, such as photosynthesis, respiration, carbohydrate and protein metabolism, translocation of substances, and growth.

A distinction should be made between atmospheric and soil drought. Atmospheric drought is characterized by a high temperature and low relative humidity of the air (10 to 20 per cent). It affects the plant by excessively increasing its transpiration. This may disturb the coordination between the rate of

water absorption and expenditure, and the plant will begin to wilt. Atmospheric drought as such may frequently be observed in spring, when the soil is saturated with water after the melting of the snow, and likewise under conditions of artificial irrigation, especially in arid climates. If the plants have sufficiently developed roots and an ample conductive system and the temperature does not exceed the limits that can be endured by plants, atmospheric drought as such is not harmful. If during the noon hours with a high evaporating power of the atmosphere the plants wilt slightly, this is of no consequence, for such wilting is only temporary. During the night, the plants recover their turgor.

Soil drought is a phenomenon much more harmful to plants. It usually takes place toward the end of the summer, when all the moisture reserves are exhausted and summer precipitation has been insufficient to replenish them. The dry soil ceases to yield water to the plant, and it goes into a state of permanent wilting, when the tissues become considerably dehydrated and growth is retarded or stops entirely. Soil drought therefore always leads to a considerable decrease of the yield. If it begins very early as a consequence of insufficient water reserves in the soil from the absence of either winter or summer precipitation, it may lead to a complete failure of the crop.

A permanent disturbance of the water balance is accompanied by changes in the physiological processes in the plant. The closing of stomata, as a consequence of deficiency of water, checks photosynthesis by impeding the penetration of carbon dioxide into the leaf. The dehydration of the chloroplasts has the same result, for their normal function is possible only when they are sufficiently supplied with water. Simultaneously, other synthetic processes are retarded, such as the formation of starch and synthesis of protein substances, while catabolic processes are accelerated. Consequently, the leaves and other organs of the plant are enriched in monosaccharides and amino acids. Increased disintegration of protein substances is caused by poor aeration, a consequence of the closing of stomata and of the checking of assimilation, which represents a source of oxygen. Oxygen deficiency contributes to the reduction of sulphydryl groups, which, as has been seen, are important respiratory activators. This leads to an increased disintegration of the proteins. Accumulation of the products of catabolism stimu-

lates in its turn respiration as well as the translocation of organic substances from the leaves. All these processes occur first of all in the lower leaves, which are more senescent and less resistant. Hence, drought hastens depletion and the death and desiccation of the lower leaves. The nutritive substances are translocated to the upper leaves and to the growing points, the existence of which is thus supported at the expense of the lower leaves.

In plants enduring permanent wilting, competition takes place between the various organs for the water reserves as well as for the nutritive substances accumulated previously. In this competition, water moves to the parts of the plant that possess the highest suction tension and greatest resistance of their protoplasm. The young leaves withdraw nutritive substances and water not only from the older leaves but also from developing fruit. Permanent wilting therefore leads in the earlier growth stages to the abscission of flower buds, to sterility, and, in later stages, to the formation of shriveled grain, called "windscorch." Windscorch will be the greater, the more vigorous the development of the leaf surface was up to the time of the beginning of the drought.

The combination of meteorological conditions unfavorable for the yield is when drought or hot dry winds in the second half of the summer are preceded by a comparatively moist spring and early summer, which contribute to a vigorous development of the vegetative organs. But moderate humidity and even a temporary drought in the first stages of development, which contribute to a less vigorous development of the vegetative organs and besides harden the plants somewhat, similar to the hardening to frost by low temperatures, represent conditions that favor resistance to drought in later stages of development. In such years, arid regions produce their highest yields.

85. Peculiarities of the Water Balance of Plants in Dry Habitats. Xerophytes, Mesophytes, and Hydrophytes.—Water is very unequally distributed over the surface of the earth. There are places where plants always have an ample supply of moisture at their disposal. But there are also large tracts of desert with a very scanty vegetation, a few isolated shrubs scattered over a naked soil. The plants of such dry habitats, where the soil contains but little water and the air is hot and dry, are called

"xerophytes." Plants characteristic of perpetually moist habitats, near bodies of water or in tropical rain forests, are called "hydrophytes." The majority of plants of temperate climates and of moderately moist habitats are of an intermediate type, the "mesophytes." A large majority of crop plants belong to this group.

Since none of these ecological types are represented by a homogeneous group, they cannot be characterized by a few definite physiological features. This is especially true of the xerophytes, plants that have adapted themselves to very severe habitats. But though exhibiting a great diversity in their adaptation, one characteristic is common to all xerophytes: They have a small transpiring surface. This feature becomes especially conspicuous when compared with the relatively large underground organs. Xerophytes are usually herbaceous plants or dwarfy shrubs, in which the underground parts are many times larger than the aerial organs (Fig. 114).

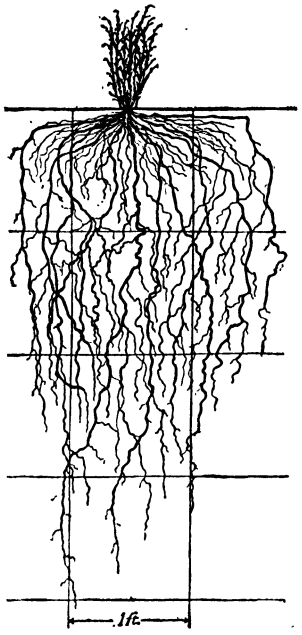


FIG. 114.—Root system of *Artemisia* (*after Weaver*).

Of the many common types of xerophytes, or desert plants, the most peculiar are the cacti (Fig. 115). They have thick fleshy stems, and their leaves have lost the assimilative function and have turned into spines. Because of their shape, the cacti combine a small surface with a considerable volume. Usually they contain large amounts of water, which is used very slowly. Experiments have shown that a cactus can thrive for several months without any external water supply. The root system of this plant spreads near the surface of the soil. During a rain, which occurs in every desert, water is absorbed quickly and collected into the stem. During the longer dry periods, the small rootlets die, and only the thicker roots remain and become covered with a corky layer. The epidermis covering the stems of the cacti has a very thick cuticle and only a few stomata, which

are almost always closed. They lose their water very slowly, even in the greatest heat. But the absorption of carbon dioxide is also checked, and cacti are distinguished by slow growth.

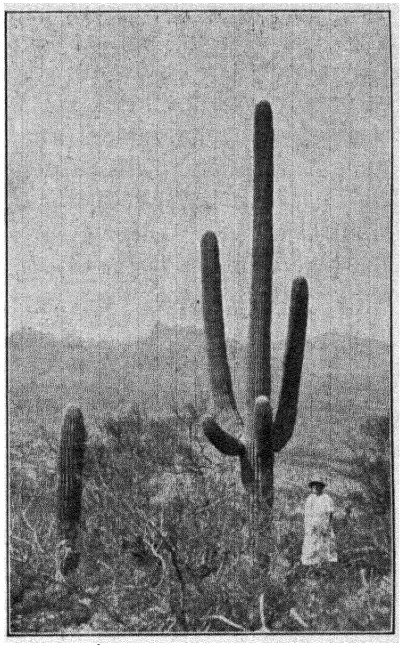


FIG. 115.—Gigantic cacti in an Arizona desert.

Physiologically related to the cacti are other typical fleshy plants, in many of which water is stored in the leaves. Such are, for instance, the aloes and agaves of the tropics (Fig. 129) and species of *Sedum* (Fig. 116) and of *Sempervivum* of the temperate zone. Such plants grow either in sand or on rocks, stone walls, roofs, and in general where the thin soil layers often dry out. All such fleshy plants are called "succulents."

Quite different from these are the thin-leaved xerophytes. To this type belong such plants as *Alhagi camelorum*, the wild alfalfa of the steppes, and some representatives of the *Cucurbitaceae*, like *Citrullus colocynthis*. Likewise, species of the sagebrush *Artemisia*, found in plains and semideserts, closely approach this group. These plants have thin tender leaves, which wilt rapidly when a branch is cut from the plant. The intensity of transpiration is very high in plants of this type, especially in sunlight. Even on very hot dry days, they keep their stomata wide open, losing considerable amounts of water but assimilating energetically. As a result of this vigorous transpiration, xerophytes of this type are able to

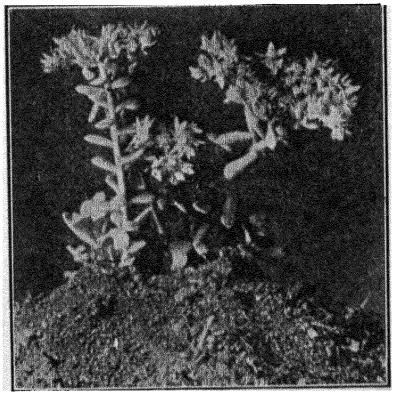


FIG. 116.—*Sedum acre.*

lower considerably the temperature of their leaves. In the hot air and soil of the desert, this protection against overheating is of great importance. The considerable loss of water is replaced from the deeper soil layers, as xerophytes of this type possess an extremely well-developed root system. Where the water table is not too deep, the plants are able to reach it with their long roots and can stand, therefore, the greatest heat. Where water cannot be reached, the root system spreads through a very great volume of soil. During the driest periods, however, water shortage commonly sets in, and these plants remain in a state of permanent wilting, throwing off a part of their leaves and even branches.

The thin-leaved xerophytes are often covered with a dense layer of gray or whitish hair. Sagebrush and many other plants of dry plains display this feature, which has often been regarded as a means of checking transpiration. This, however, is an erroneous conception, as experiments have shown that the intensity of transpiration is very high in such plants. These hairs must be looked upon as forming a light screen to protect the plant against overheating and the chlorophyll apparatus against too bright light. They also serve as a protective covering against the attacks of animals, as do the spines and the bitter, astringent, and pungent substances that the plants often possess. An important physiological peculiarity of xerophytes of this type is the high concentration of the cell sap, which enables the wilting plant to develop an enormous suction tension and to draw water from the soil very rapidly. Moreover, it is possible that the soluble substances that accumulate in large amounts in these plants make them more resistant to permanent wilting.

The third type of xerophytes are sclerophyllous plants with rigid leathery leaves. To this group belong many prairie grasses, such as *Stipa* and *Festuca* and some *Umbelliferae* such as *Eryngium*. The sclerophyllous xerophytes are especially widely distributed in the dry districts of the Cape Province, Africa, where may be found many representatives of the family *Ericaceae*. These plants are characterized by an unusual capacity to resist permanent wilting. The margins of the leaves commonly curl inward, and the stomata are hidden in a tubelike depression (Fig. 117). They are able to preserve for a long period the last portion of water indispensable to life. The physiology of these

plants has been investigated very little. The sap concentration of the cells is very high in xerophytes of this type, while in the succulents it is, on the contrary, very low.

In addition to these groups of true xerophytes, which are able to withstand in one way or another the driest and hottest weather, there are found in the desert also plants known as pseudo-xerophytes. They are the ephemeral plants, characterized by an extremely rapid development. Within the span of 3 to 4 weeks, these plants will germinate, bloom, set fruits, produce mature seeds, and die. Their whole life cycle occurs in the short moist period, usually in spring. During the remainder of the year, they exist in the form of dry seeds that cannot be affected by desiccation. In all their other characteristics, these plants are typical mesophytes.

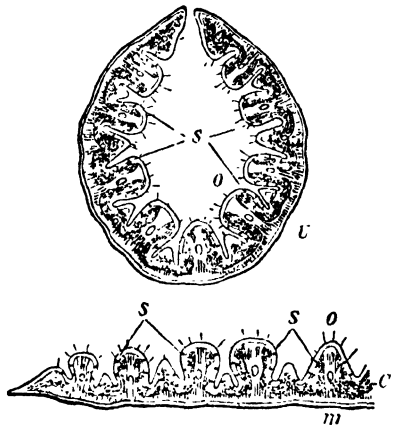


FIG. 117.—Leaf of *Stipa* curled into a tube, *U*, open *M*.

The groups discussed by no means exhaust the various xerophytes. The types sharply delimited here, moreover, are connected by a series of plant forms showing imperceptible transitions from one group to another. A detailed examination of these types belongs to the field of ecology.

86. Effect of Environment on the Structure of Transpiring Organs. Xeromorphism and Drought Resistance of Plants.—The transpiring organs of plants, which in practically all cases are the leaves, exhibit a considerable variability. Leaves of different plants and even of the same plant show considerable differences in structure, depending on the external environmental factors under which they develop.

A very important regularity in the structure of the leaves, depending upon their position on the plant, has been established by Zalensky (1904). He has found that leaves on the upper half of a stem are always different from those below. The higher the position of a leaf, the smaller are the dimensions of all its cells, but the greater the number of stomata per surface area,

the size of each stoma being smaller. Terminal leaves have also a thicker network of vascular bundles, a greater number of hairs per surface area, and a thicker layer of palisade tissue. This dependence of structure on position has been called "the rule of Zalensky." Figure 118, showing the vascular network in an upper and lower leaf of tobacco, may serve as illustration of this rule.

These anatomical peculiarities may be correlated with physiological differences. The upper leaves are distinguished by higher assimilation and more intense transpiration. The osmotic pressure of their cells is higher, and in wilting the upper

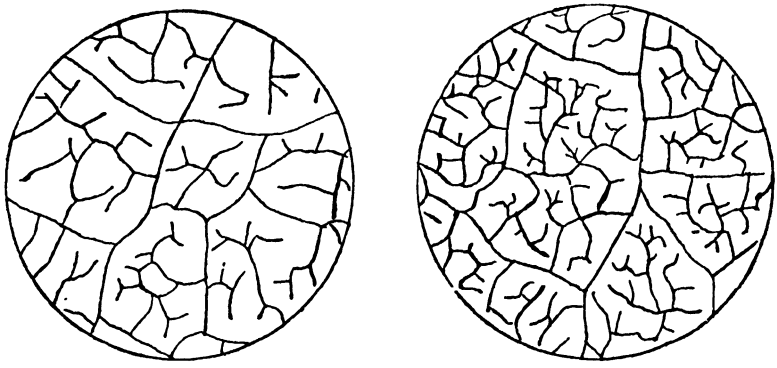


FIG. 118.—Vascular network in a lower (left) and an upper (right) tobacco leaf (*after Zalensky*).

leaves draw water from those lower down. Thus when permanent wilting takes place, the lower leaves will release all their water and die. In case of water shortage, stomata of the upper leaves remain open longer and thus may continue to assimilate over a greater period. As these are characteristic features of many xerophytes, this structure is called "xeromorphism."

These differences in the structure of the upper leaves may be due to the fact that they develop under the conditions of a somewhat lower water supply, which may result in smaller dimensions of their cells. The same xeromorphic structure may be induced by the direct influence of external factors on the plants as a whole, such as increased dryness of the air, reduced soil moisture, and periodic wilting. Plants developing under such conditions are smaller in size but have a greater drought resistance.

Similar changes are produced by increased light intensity. Generally, sun leaves differ from shade leaves by the same characteristics as do the upper leaves from the lower ones. Especially marked are the changes in the development of palisade parenchyma, as has been seen in Art. 45. Among the physiological characteristics of sun leaves may be mentioned an increased assimilation, at least in strong light, and an intensive transpiration. Consequently, sun leaves may be considered xeromorphic leaves.

The study of the anatomical and physiological peculiarities of xeromorphic structures is of great importance in the determination of differences in drought resistance among varieties of crop plants.

Formerly, it was assumed that the capacity of a plant to expend the least possible amount of water is the basic feature of drought-resistance. Hence, it was concluded that low transpiration intensity and a low transpiration coefficient were the most reliable physiological peculiarities of drought-resistant plants. However, the basic contradiction in the life of land plants was omitted, which has been indicated in Art. 64 and according to which a decrease in water expenditure inevitably leads to a change for the worse in the conditions of both aerial and mineral nutrition of the plants. It has already been seen that in the xerophytes of the deserts this contradiction is solved in different ways. In drought-resistant crop plants, likewise, it is solved, but differently. There is not a uniform type of drought resistance, just as there cannot be universal features of drought resistance.

As crop plants, unlike the desert xerophytes, are not subject to drought throughout their vegetative period but only during more or less short intervals of time, one of the most important features of drought-resistant plants is their capacity of enduring temporary dehydration of their tissues without decreasing, or with the least decrease of, their yield (Maximov, H. Walter). Thus for instance, according to Tumanov, permanent wilting caused by drying of the soil does not affect the yield of millet, a plant which is very drought resistant, while it sharply decreases the yield of oats. The capacity of enduring without injury the loss of a considerable part of their water allows drought-resistant plants to keep their stomata open and to continue assimilation even during periods of intense drought. Wassiliev has been

able to show that one of the important features of drought resistance, at least in wheat varieties, is the diurnal changes of the stomatal movements. In the dry climate of Southeastern Russia, the resistant South-Russian wheat varieties keep their stomata open all day long, while the less resistant Canadian varieties close them early in the morning and thus starve and produce a low yield. The capacity of enduring wilting is probably connected with an increased content of osmotically active substances and hydrophilic colloids in the cells of drought-resistant plants. With sufficient water supply, the leaves of drought-resistant wheats have a higher water content than those of nonresistant varieties (Litvinov).

Although the capacity of enduring considerable dehydration of their tissues without injury is one of the essential features of drought-resistant plants, nevertheless the drought resistance of all varieties and crops cannot be explained by this one feature. Anatomical and morphological peculiarities, such as a well-developed root system and the storage of water reserves in fleshy stems and roots, may also be of great significance. Of no less importance is the xeromorphic structure, which has been examined above. Hence in selecting drought-resistant varieties, it is important to pay attention to the size of the plant cells, as has been indicated by Kolkunov (1904). The possibility of the plant withstanding drought by means of various physiological and anatomical adjustments makes it insufficient to determine the degree of drought resistance on the basis of a single feature, even if it is a feature of major importance. It is more reliable to apply the more complicated methods of direct testing of the resistance to different forms of drought, especially to soil drought and to hot dry winds. To accelerate this testing, which up to the present time was carried out under field conditions, different forms of desiccators, dry wind chambers and other devices, have been devised, which allow one to subject the plant to any combination of soils and atmospheric factors (Shirley, Aamodt).

In most arid regions, the soil dries completely toward the end of the vegetative period in spite of the scant summer precipitation. Early varieties in such regions produce higher yields than late varieties, for they complete their life cycle and ripen their grain before the complete consumption of all the available moisture. Consequently, in the practice of agronomy earliness is

very often confused with drought resistance, and varieties that ripen in time to evade drought are generally accepted as drought resistant. The method commonly adopted for the determination of drought resistance in field variety testing contributes to this confusion of concepts. The yield of varieties is compared in dry years, and those that are the most productive are accepted as most drought resistant. It is the early varieties that produce a higher yield when there is a late drought.

Plants show a different resistance to drought at various growth stages. In cereals, there is the greatest depression when they are subjected to drought at the time of the formation of the floral organs. Withdrawal of water by the leaves in this period stunts the developing embryo and leads to alternate setting or to poor filling of the grain. This sensitive period has been designated the "critical period." It was first observed by agrometeorologists (Brownov, Hauer, Azzi) by means of comparing yield with the time of the summer precipitation. Later on, the existence of critical periods was proved by means of physiological experiments.

The complexity of the conditions and the existence of different types of drought resistance make the selection of plants difficult. Plants cannot be chosen according to one peculiarity, as for instance the intensity of transpiration or the coefficient of transpiration, as it has been customary to do. First of all, there must be established the factors of drought that are found in the various geographical regions, as has been indicated in the case of winter resistance. For instance, one should know the prevalence of soil and atmospheric drought; the time of its manifestation, whether in spring or summer; and finally, the reaction of the different varieties to the different types of drought. As yet there are no satisfactory methods of directly determining drought resistance that are as reliable as the methods of determining frost resistance by means of direct freezing. This hinders selection work and compels recourse to field methods, which require a long time and do not always give clear results. At present, the wilting method suggested by Tumanov is most widely used. Plants are grown in pots and at definite stages of their development are subjected to permanent wilting, by stopping watering for some time. The decrease in yield of the wilted plants as compared with the control plants serves as an index of their

drought resistance. But as the capacity of enduring permanent wilting is not the only index of drought resistance, the method of wilting does not give reliable results.

87. Physiological Basis of Irrigation.—In hot arid regions, the precipitation of the period of vegetation together with the moisture reserves remaining from winter is insufficient for the cultivation of crop plants. The only means of raising the productivity of such land for rearing cattle that feed on the wild xerophytic vegetation and for cultivating crops is the application of artificial irrigation. In such dry regions as are sufficiently provided with rivers that carry water from mountains or from more humid regions, artificial irrigation of the field has been applied from prehistoric times. But even in parts of the country where dry farming is possible but where sufficiently high yields cannot be obtained, the application of artificial irrigation is of great public significance as one of the most reliable methods of the control of drought that occurs periodically in such regions.

The technique of the irrigation of large tracts of land differs widely from the methods of watering of small vegetable gardens. One of its chief purposes is a periodic deep moistening of the soil under cultivation, to form a water reserve that will supply the plants during several weeks. In irrigation, either the surface is flooded with water, which is gradually absorbed by the soil; or, if the field is situated on a slope, water is allowed to flow along the surface or through special furrows between the plants until the soil is moistened to a sufficient depth, at least 70 to 100 cm.

During the application of water and immediately afterward, the plants are in a saturated soil; but during the comparatively long interval between irrigations, they gradually consume all the moisture, and toward the end of this interval, the soil is usually quite dried. Hence, plants developed under periodic irrigation have to endure drought that somewhat retards growth. The longer the intervals between irrigation, the greater this retardation. With very long periods between water applications or with too early cessation of irrigation, which may be caused by insufficiency of irrigation water or the absence of a systematic planning of its distribution, the plants, overwatered during the early irrigations, may be in worse condition than those that have been hardened in the beginning of their vegetation.

Frequent irrigations are most favorable for the development of plants, but the amount of water applied at every irrigation must be decreased, because an excess of irrigation water above that indispensable for the plant produces a harmful effect. It compacts the soil excessively and sometimes even creates a condition of waterlogging that decreases the development of the roots. The chief harm from excessive irrigation is the percolation of the irrigation water into the deeper soil layers, which are frequently rich in soluble mineral salts. The rise of the water table accompanying overirrigation promotes the capillary movement of water together with the soluble salts toward the upper soil layers, thus increasing a concentration of alkali in them. Irregular use of irrigation water very often is the cause of too much alkali in soils. Many examples may be mentioned where large tracts of fertile soils with expensive irrigation systems have been abandoned because of an excessive concentration of alkali in the top soil.

The time of irrigation, its frequency, and the amount of irrigation water must be planned in such a way as to allow the plants to consume all the water applied, during the interval between the irrigations. The application of water, however, must not be planned purely mechanically according to the calendar; it must be coordinated with the development of the plants, so as to provide sufficient water during the critical periods. For spring wheat, for example, it is indispensable to supply the plant with abundant water during the period of shooting and later during the filling of the grain. Wheat is usually irrigated twice: the first time, toward the end of the tillering phase, before shooting; and the second time, soon after flowering, before the beginning of filling of the grain.

Very desirable from a physiological point of view is the new method of spray irrigation. The water is directed onto the field through portable pipes terminating in spray nozzles, which discharge water over a large surface area in fine drops similar to rain. This method makes it possible to apply water in small amounts, corresponding to 2 to 3 cm. of precipitation, while the usual methods of surface irrigation require a layer of water no less than 8 to 10 cm. to guarantee a uniform distribution of water on the field. Spray irrigation thus makes it possible to maintain the soil in a sufficiently moist state, eliminating the

usual period of drought in the intervals between irrigations and at the same time not giving too large amounts of irrigation water at a time.

88. Injury to Plants from Excessive Humidity. Factors in the Resistance of Bog Plants to Waterlogging. Lodging of Plants, Its Causes and Means of Control.—An excess of water in the soil may be almost as harmful as a deficiency; waterlogged soils can be used for cultivation only after drainage. This is not because water is harmful to the roots; for experiments with growing various plants, even xerophytes, in water culture have proved that this is not true. But when the soil is waterlogged or temporarily flooded, all the capillaries are filled with water, and air cannot penetrate into the soil. The roots are deprived of the oxygen indispensable for their life activity. It has already been emphasized that oxygen is one of the conditions essential for water absorption by the root that supplies water to the aerial parts (Art. 65) and likewise for the absorption of mineral and nitrogen salts (Art. 58).

Besides this direct influence of oxygen deficiency upon the roots, the filling of the soil capillaries with water and the decrease of aeration in general lead to a series of other very serious consequences. The most important of these is the checking of the normal oxidation in the soil produced by the activity of aerobic soil bacteria. Anaerobic processes then begin, chiefly butyric and similar fermentations. Carbon dioxide and other organic acids accumulate in the soil, as well as the products of reduction both of organic and of inorganic substances, many of which are toxic to the roots of plants. American authors term these products "bog toxins." Crop plants, whose root systems require a loose, well-aerated soil, cannot grow on such soils. Only special bog plants can thrive on them, their roots being specially adapted to these conditions and unsusceptible to the bog toxins. They have anatomical structures that provide them with oxygen for respiration, even in soils that are completely lacking in oxygen. The stems of bog plants frequently possess well-developed intercellular spaces and air cavities that are connected with similar cavities in the stems and leaf peduncles. Oxygen is formed in photosynthesis and, expanding with the heating of the leaves by the sun's rays, is forced

through these cavities into the roots, where it is consumed in respiration.

Only a few of the crop plants can grow on waterlogged soils. Rice is the most important of these. It not only endures but even requires for successful development flooding of the field with water during the greater part of its vegetative period. Most of the plants, however, can grow on bog soils only after drainage, which causes a lowering of the water table and is accompanied by the penetration of oxygen at least into the upper soil layers. Anaerobic processes are then replaced by oxidation processes, the toxins are destroyed, and the roots of plants can develop normally. All these changes require a long time. Bog soils become fit for cultivation of crop plants only 1 or 2 years after drainage.

One of the results of the anaerobic fermentation is the accumulation of an excessive amount of organic acids and substances that increase the hydrogen-ion concentration. Bog soils are frequently very acid. Plants inhabiting them, such as species of *Carex* and *Deschampsia caespitosa*, are characterized by a very high pH optimum of their growth. After drainage of bogs, soil acidity decreases slightly but not sufficiently. Hence, liming of drained bog soils is essential. It improves the reaction and contributes to a more normal decomposition of organic soil compounds.

With a temporary excess of soil moisture, for instance, in the spring when snow melts or during prolonged rains that make ponds in depressions of the field surface, injury to plants from an excess of water may occur. The results in this case are similar to those occurring in bog soils and are explained by insufficient penetration of oxygen into the soil. Temporary waterlogging retards somewhat the growth of plants and weakens them in their struggle with weeds, which may develop vigorously under conditions of excessive soil moisture. Prolonged waterlogging leads to death of crop plants.

Lodging of cereals, which also frequently results in considerable losses, is likewise to a certain degree caused by excessive soil moisture as well as by high atmospheric humidity. Lodging is frequently observed in rainy summers and with artificial irrigation. The chief cause of lodging is the disturbance of the equi-

librium between the weight of the spike and the resistance of the culm that carries it. Insufficient thickening of the culm and a weak development of its mechanical elements are often the consequence of excessive tillering and overdevelopment of the leaves caused by an abundant supply of water, which leads to a thick stand and shading of the plants. Deficiency of light promotes an excessive elongation of the shoots and insufficient development of the mechanical tissues, thus creating conditions that favor lodging.

Likewise, lodging may be stimulated by excessive fertilization, especially with nitrogen. Nitrogen contributes to a vigorous development of the vegetative organs, as well as to an excessive increase in the size and weight of the spike. Lodging is usually produced during occasional strong winds or rains. The weight of the spike is increased by the water held on the culm and leaves, and the stem bends over at its base. Lodging is more serious if the soil is saturated with water. The wet soil is not firm enough to hold the roots that maintain the culm in an upright position.

The ability to bend at the nodes, seen in the culms of cereals, allows the lodged plants to raise their spikes and to recover from the harmful consequences of lodging. However, the yield is always somewhat decreased, especially if the field is infested with weeds. Cutting and binding lodged and entangled grain is very difficult, and the losses during harvesting are greatly increased. There are certain methods used for the control of lodging. The plants are sown less thick; the application of nitrogen fertilizers is reduced; and excessive irrigation is avoided. Under conditions where lodging may be expected, varieties that possess a firm culm and that are resistant to lodging should be chosen.

89. Injury to Plants from Excess of Salts. Physiological Peculiarities of Halophytes. Alkali Resistance of Crop Plants.—In regions of low annual rainfall, the amount of water precipitated is insufficient to wash out all of the mineral salts released in the processes of weathering, and the soils gradually accumulate them. The rain water flowing into depressions accumulates the salts from the surrounding surface, and evaporation leaves the salts in the soil. Such an excessive accumulation of salts is harmful to most crop plants, and in such depressions and salty spots they develop less vigorously. Extremely large accumulations of salt on so-called "alkali soils" makes them quite

unfit for the cultivation of crop plants. Such saline lands are inhabited by especially salt-tolerant plants, the "halophytes," which are distinguished from usual plants by anatomical and physiological peculiarities.

Excessive salinity of the soil is injurious to the plant in two ways. First, the accumulation of salts leads to an increase in the osmotic pressure of the soil solution. Consequently, its water-supplying power is decreased, and the water supply to the plant is reduced. It has already been noted that the suction tension of the roots is not high, and an increase of the osmotic pressure of the soil solution up to 5 or 10 atmospheres, corresponding to a 0.6 to 1.2 per cent solution of sodium chloride, creates a serious hindrance to absorption. Experiments show that the total concentration of salts in water cultures must not exceed a few tenths of 1 per cent and must not have an osmotic pressure of over 1 to 2 atmospheres. Watering the soil with salt solutions likewise causes a rapid wilting of the plants as a result of a check to the normal water supply.

Second, the excessive accumulation of soluble salts has a toxic effect upon the plants. Even such salts as sodium chloride and sulphate, which are widespread in saline soils and which in weak concentrations are neutral, at higher concentrations are toxic and upset the normal physiological functions. Magnesium salts and the alkaline carbonates that usually accompany sodium salts are especially toxic. In the discussion of the mineral nutrition of plants, the injurious effect of the various anions and cations has already been noted. Though in saline soils the salt solutions are usually balanced, at high concentrations their toxic effect becomes apparent. But different plants show varying degrees of tolerance in this respect.

Under natural conditions, alkali soils are inhabited by a relatively rich and diverse vegetation consisting of halophytic plants well adapted to high salt concentrations. According to the investigations of Keller as well as of other authors, the halophytes may be divided into several sharply distinct groups. Plants of one of these groups are very resistant to salts and accumulate them in their cells in large quantities, thereby increasing considerably their osmotic pressure. This group is formed mostly of *Salicorniaceae*, which develop on saline soils on the shores of seas and salt lakes and near salt deposits. The *Salicorniaceae*

are distinguished by a number of anatomical and physiological peculiarities. These plants often possess fleshy stems and leaves and resemble the cacti (Fig. 119). This characteristic structure is hereditary, for it is reproduced by the plants when grown on soils where no salts are present. With an increase of the salt concentration in the soil, succulence increases, which after all may be regarded as a measure, or as one of the direct results, of high soil salinity. Because of the abundance of sodium chloride in some saline soils, large quantities of this salt are found also in the ash of plants native to such soils. Sodium chloride is not an absolute physiological necessity for saline plants. They can grow on soils that are not saline. The experiments by Keller, however, have shown that the addition of this salt improves their development (Fig. 120). This is one of the reasons why they are able to displace from alkali soils all the other plants, most of which are injured by a certain concentration of salt. Excessive amounts of sodium chloride, however, can injure even the most tolerant of saline plants.

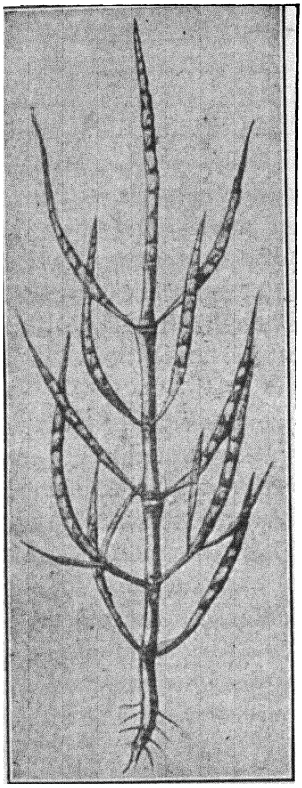


FIG. 119.—*Salicornia*, a fleshy plant from saline soil (*after Schimper*).

Another group of halophytes is formed of plants for which accumulation of salts in the cell is almost as toxic as for common mesophytes and the roots of which possess a low permeability to salts. They attain the suction tension necessary for water supply by means of increasing osmotically active organic substances such as organic acids and sugars. This group includes various species of the sagebrush, which covers large tracts of the saline semi-deserts, and a number of other plants. This group grows on less saline soils than *Salicornia* does.

The third group is made up of plants whose root cells, like those of the *Salicorniaceae*, are permeable to salts but which do

not accumulate them in their stems and leaves, possessing special adaptations for removing them. They are provided for this purpose with numerous secretive glands covering the surface of their stems and leaves. *Statice* and the shrub *Tamarix*, widely distributed in the steppes and deserts, belong to this group. In dry weather, a deposit of salts completely covers their surface and is partly blown away by the winds and partly washed off by rain.

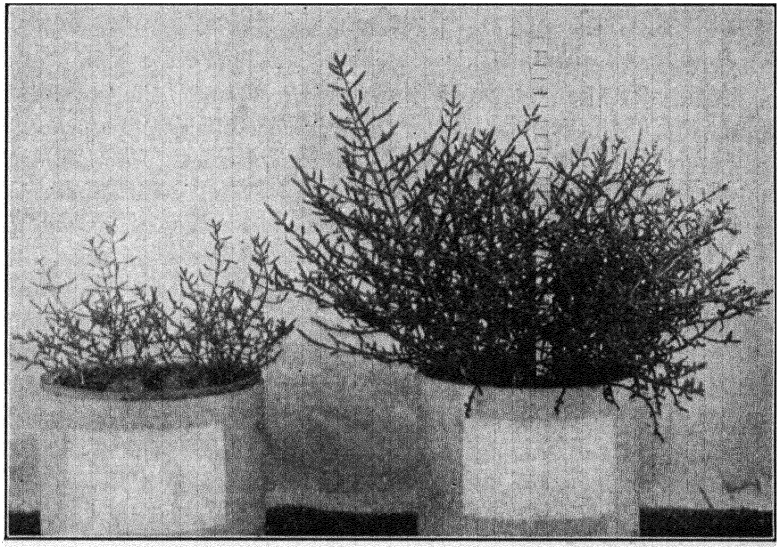


FIG. 120. *Salicornia* plants growing without sodium chloride, on the left; and with the addition of sodium chloride, on the right (*after Keller*).

Among the crop plants, true halophytes are not found, just as true xerophytes are not met with, but there are more or less salt-tolerant forms among them. Cotton, alfalfa, beets, tomatoes, and water melons, for instance, tolerate a certain accumulation of salts in their tissues. Soft wheat varieties are more salt resistant than hard wheats. Flax, oats, and buckwheat are not salt resistant. Within the limits of each crop, more and less resistant varieties can be found; but this feature has been very little studied, and no systematic work has been done as yet on selection of salt-tolerant varieties.

The harmful effect of soil alkali is manifested on the plant in different ways. The high concentration of the soil solution impedes the swelling and germination of seeds as well as the

growth of the root system of the seedlings. As a result, plants are produced that remain backward in their development and are seriously injured from drought.

The accumulation of salts in the cells, resulting in poisoning of the protoplasm, retards the synthetic process, such as photosynthesis and the function of proteins. The cells accumulate chiefly soluble carbohydrates, amino acids, and such soluble compounds. Their accretion increases the osmotic pressure in the cells, which retards the growth of plants, though it is advantageous from the point of view of obtaining water from more concentrated soil solution.

Salinity of the soil has a singular effect upon the stomatal apparatus of the plant. Under the influence of salts, the process of starch formation is impeded in the guard cells. As has been noted above (Art. 71), the formation of starch contributes to their normal closing when water is deficient. Hence, on saline soils the stomata are open permanently even during drought. This results in an excessive water loss and threatens the plant with desiccation. But at the same time this effect of salts, which contributes to the accumulation of soluble carbohydrates and similar compounds, increases the drought resistance of plants, thereby compensating to a certain degree for the loss of water.

Salinity of the soil has a great influence upon the filling of grain, interfering with its development and causing it to shrivel. Accumulation of carbohydrates is retarded to a greater degree than is that of protein substances. As a consequence, on saline soils wheat produces grain rich in protein. This increases considerably its commercial value.

90. Resistance of Plants to Fungi. Character of the Interrelations between the Parasite and the Host Plant. Immunity.—Among the factors causing considerable decreases in the yield, besides drought, frost, and alkalinity, there may be mentioned the attacks of parasitic fungi. Rusts, smuts, root rots, and other diseases cause enormous damage in agriculture, and the struggle with them represents one of the essential problems in present-day agriculture. A detailed discussion of all these diseases cannot be made in this textbook. Only a few of the most important facts of the physiology of fungus diseases will be considered here. A description of the symptoms of diseases and of the fungi causing them is the subject of phytopathology.

The interrelations between plants and the fungi that infect their tissues and feed upon their sap are usually termed "parasitism," the parasite causing more or less harm to the host plant.

Injury is caused by different vital activities of the parasite. Often it is due to the withdrawal of nutritive substances from the host plant, leading to its exhaustion. However, such a direct injury is usually quite insignificant; for the green plant forms organic substance with ease and in abundance, while the total size of the parasite may be very insignificant compared with the host plant, and thus the amount of substances it consumes cannot cause great depletion of assimilates. More serious are the disturbances of the normal physiological functions caused by the penetration of the parasite into the tissues of the host plant. It has been observed frequently that under the influence of fungi, the intensity of respiration increases considerably, resulting in greater expenditure of organic substance. Sometimes this increase in respiration amounts to 15 to 30 per cent, but at other times it reaches 200 per cent. Such a rapid expenditure of substance inevitably leads to an appreciable exhaustion of the host plant and to a decrease of the yield.

Affecting the leaves of the plant and injuring in particular the chlorophyll-bearing tissues, many of the parasitic fungi considerably decrease the intensity of photosynthesis of the leaves. This decrease often attains 25 to 50 per cent; and, in some cases, when the parasite develops very abundantly, it may be even 70 per cent and more (Kuprevitch, 1934). Such a decrease in photosynthesis together with the increase in respiratory intensity considerably depresses the growth of the affected plants and creates unfavorable conditions for the development and filling of the grain. An epidemic of rust causes the same effects as wind burn and windscorch; *viz.*, the grain is shriveled and poorly filled. In some instances, however, when the infection is not great, a fungous infection may produce stimulation that leads not to a decrease but to an increase of the intensity of photosynthesis. In such cases, infection does not hinder the plant, and the relation between the parasite and the host plant approaches symbiosis. As has already been emphasized in the discussion of the interrelations between the leguminous plants and the nodule bacteria, the boundary between parasitism and

symbiosis cannot be sharply delimited. The parasite infecting the tissues of a plant in some cases depresses it; in other cases, it stimulates the host plant. Frequently, if the infection does not surpass certain limits, it does not appreciably affect growth and development of the host plant.

Infection with fungi likewise considerably influences the water balance of plants. Injuring the protective tissues and frequently filling the stomatal openings with their hyphae and thus hindering their closure, the parasitic fungi usually contribute to a considerable increase of transpiration. This is of little consequence in a humid climate; but under conditions of drought, the loss of the capacity of controlling stomatal movements and the injury to the cuticle may be very harmful.

The injury is still greater when the conductive tissues are blocked. This occurs where the fungal hyphae fill the lumens of the vessels and tracheids and hinder the ascent of water. The result of such an infection, frequently caused by *Fusarium* and other closely allied genera, is the wilting of plants under conditions apparently most favorable for water supply. Such diseases often cause great damage in fields of sunflower, cotton, etc. Infectious wilts most frequently strike young seedlings, but sometimes mature plants, apparently healthy, succumb to this disease.

A very widespread type of injury is the poisoning of cells and tissues by toxic substances excreted by the fungal hyphae. Such poisonous substances are designated by the general term "toxins." As has been shown by the recent investigations of Schaffnit (1932), toxins are excreted by the fungi into the surrounding medium. Penetrating into the conductive tissues of the plant, they may cause the poisoning and wilting of the tissues and organs, chiefly leaves and young stem tips to which they are carried by the transpiration stream. According to Schaffnit, the cause of infectious wilt, in particular the disease caused by *Fusarium*, is intoxication of the tissues and not blocking of the conductive vessels. His investigations have proved that the toxins represent nonvolatile crystalline substances, not inactivated by boiling, with an alkaline reaction, and approaching in their properties diamino acids formed during the hydrolysis of proteins. Their action is not specific The same toxin is equally poisonous to different plants.

So long as the plant is capable of resisting infection, it combines the toxins excreted by the fungi with protein substances that it elaborates. This phenomenon may be observed, for instance, in the case of infection with rust. Owing to this capacity of binding toxins, the disease is very often localized in definite tissues, which are frequently separated from the healthy parts of the plants by a cork layer. From this combined state, the toxins may again be liberated by the activity of proteolytic enzymes.

The penetration of the parasite into the tissues of the plant may be accomplished in various ways, but most often the paths of infection are the stomatal openings on the aerial parts and the root hairs. Infection rarely occurs directly through the surface walls of the epidermis; for they are usually thickened and besides covered with a layer of cuticle, which is very impervious both chemically and mechanically. When the hyphae of the parasite penetrate into the leaf through the stomatal openings, they come into direct contact with thinner and more permeable walls of the parenchyma. But there exists a group of fungi, the mildews, which have the ability to perforate by means of appressoria the thick outer walls of the epidermis though covered with the cuticle. Formerly it was assumed that the cell walls of the host plant are dissolved by a specific enzyme, but recent investigations (Hawkins and Harvey) have shown that the walls are perforated mechanically by means of the high turgor pressure in the hyphae of the fungi. In the roots, infection takes place through the cells of the root hairs, which possess very thin cell walls. The older parts of the roots, which have shed their root hairs and are covered with a layer of cork, are well protected from the intrusion of the parasite. In young seedlings, the infection frequently penetrates through the crown, *i.e.*, the region between the root and the stem, which is situated near the surface of the soil. This method of infection may frequently be observed in hotbeds. When the stand of the seedlings is too thick, the walls of the cells are thin and offer little resistance to the entrance of parasites.

The ability of plants to resist diseases is generally termed "immunity." There are different types and different causes of immunity. Sometimes resistance is based on anatomical adaptations, for instance, on the presence of a thick cuticle such as

may be found on winter varieties of apples or on the early formation of cork tissue. Strong lignification of the vessel walls inhibits the penetration of the mycelium of *Fusarium* causing wilting. Such immunity is designated "passive" and may be easily lost through mechanical injuries, for instance as when the skin of fruit is pierced by insects. Another form of passive immunity is shown when the chemical composition of the plant does not suit the requirements of the parasite, which consequently cannot develop successfully, though its spores may be able to germinate. This is a very widespread form of immunity, for most of the parasitic fungi as a rule show very narrow specialization in their nutrition and require definite nutritive substances. They are adapted to certain host plants that contain the strictly necessary substances.

By "active" immunity is understood the elaboration by the plant of substances that make the development of the parasite impossible. Formerly it was assumed that such an almost universal mechanism of immunity was the change of the actual acidity of the cell sap in a direction unfavorable to the parasite, commonly by increase of the acidity, but later it was found that the parasitic fungi are capable of a rather wide adaptability to the reaction of the medium. Likewise it was often assumed that an increase in the concentration of the cell sap, inhibiting the growth of the fungal hyphae, represented one of the factors of immunity, but this feature is far from being universal. More detailed investigations have shown that there do not and cannot exist universal causes of immunity. Plants possess various factors of immunity to different parasites.

Comparisons based on the analogy between the infection of animals and plants gave rise to the idea that weak poorly nourished plants are especially susceptible to fungous diseases, but this idea has not been confirmed. This viewpoint is to a certain degree correct in respect to attacks of harmful insects, for instance, certain plant lice, which frequently attack weakened or exhausted plants, and likewise weakly parasitic fungi, which may become strongly parasitic on weakened hosts. Fungous parasites, especially rust, on the contrary, attack more readily the vigorous healthy plants. Fertilizing the soil with nitrogen and phosphorus may increase the infection. The introduction of potassium fertilizers slightly decreases rust infection. Like-

wise, varieties rich in certain vitamins are usually readily infected by parasites, which do not have the capacity to elaborate them though they are indispensable for their growth (Suchorukov). Infection is stimulated by activity of the oxidation processes, and it is sometimes possible to establish a parallelism between infection and the activity of peroxidase and other oxidation enzymes (Richter).

A detailed discussion of the phenomenon of resistance to fungal, bacterial, virus, and other infectious diseases lies beyond the scope of a course in plant physiology.

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CHAPTER XI

INTERRELATIONS BETWEEN DIFFERENT PARTS OF THE PLANTS. VEGETATIVE PROPAGATION

91. The Plant as a Unit Organism. Correlation.—Separate parts of the plant possess a considerable degree of independence, much greater than the separate parts of the animal organisms. The well-known method of propagation by means of cuttings, which will be discussed in detail further on, shows that a small section of a branch of a plant, provided that it carries a bud, will frequently reproduce all the rest of the organs of the plant. In some plants, even small bits of a leaf, stem, or root when separated from the mother organism may form a new growing point and reproduce the whole plant. There are reasons to suppose that any undifferentiated cell of a higher plant should be capable of regenerating the whole organism. If this does not always occur, the chief reason is that all the conditions that are necessary for regeneration and that are not yet known have not been fulfilled.

This independence of the separate parts of the plant serves as a basis for some of the methods of study of physiological processes. In the discussion of respiration, photosynthesis, transpiration, and other functions of the plant, it has been seen that very often they are studied on separate branches and even leaves, the supposition being that the parts of the plant represent the whole and that there is no substantial difference in the processes occurring therein. This has led many to the conviction that the plant does not represent a united whole but resembles a colony of separate parts very little connected with each other.

This concept is quite erroneous. In spite of the clearly understood ability of the parts of plants to continue an independent existence after separation, the plant represents a unit structure, all the parts of which are reciprocally coordinated and produce a certain influence on each other. Cases of such a coordination have already been cited. For instance, in the discussion of the water balance of plants, it has been shown that

the absorption of water is dependent upon the suction tension of the transpiring leaves and, conversely, that the intensity of transpiration depends on the conditions of the absorption of water by the roots. The absorption of salts by the roots of plants depends on the photosynthetic activity of the foliage and in turn upon the elaboration of pigments in the leaves. The utilization of photosynthate in growth is dependent upon the absorption of nitrogen and mineral salts.

Numerous examples of such reciprocal assistance by separate parts of the plant could be cited, but the interrelations of organs find their expression likewise in other directions, for instance, in their mutual relations in development.

The influence of the growing point upon the lower buds may serve as a striking example of growth correlations. In many plants, as for instance the sunflower, the stem does not branch, though a bud is formed in the axil of each leaf. This bud remains during the whole life of the plant in a dormant state; after maturing of the seed, the whole plant dies, together with the buds that have never manifested their capacity for further growth and development. But as soon as the top of the growing stem is cut, a few, or all the dormant buds begin to develop rapidly into lateral shoots, each of which terminates in an inflorescence that develops a number of seeds. This compensates somewhat for the loss of the large inflorescence that should have terminated the central axis.

The influence of the top on the lower parts of the plant is very clearly demonstrated in trees, especially in conifers, which are distinguished by a very regular branching. For example, the trunk of the fir tree terminates in a top bud surrounded at its base by a crown of four to five lateral buds. In spring, shoots develop from these buds, the main shoot growing exactly vertically owing to its negative geotropism, while the lateral shoots grow out at a definite angle to the main axis.

If the top bud is injured, this directly influences the character of the growth of the lateral shoots. Some or all of them begin to grow vertically. The main axis is thus either replaced by a shoot that formerly was a lateral bud, or several vertically growing shoots may develop. If the entire top of the fir tree is removed with all its buds, the functions of the main shoot are transferred to one or several of the lateral branches, and their

tops, which previously grew obliquely, now begin to grow vertically.

Such instances of reciprocal influence of parts of the plant on the character of growth have been generally termed "correlations." The nature of these phenomena for a long time remained doubtful and this gave occasion for different sorts of vitalistic interpretations. It may be assumed that correlations are phenomena of a hormonal character, *i.e.*, that growth is regulated by means of specific chemical agents. According to this concept, the top of the stem elaborates special substances that inhibit cell division in the meristematic tissues. Thus they likewise retard the growth of the lateral buds and retain them in a "dormant" state. With the removal of the top, the stream of this inhibiting substance is checked, and the lateral buds begin to develop rapidly. According to recent data, this substance is identical with the growth-promoting substance auxin (see Art. 29).

An analogous correlation may be observed in the development of the root system. The growing root tip inhibits the formation of laterals near the tip, and branching always begins at a considerable distance from the end. If the root tip is removed, immediately an acceleration in the initiation and development of the laterals will take place. This phenomenon is well known in the practice of horticulture and vegetable gardening. Transplanting seedlings serves not only to give each plant a larger root surface but also changes the character of the growth of the root system. In transplanting, the main root may be cut off and replaced by lateral rootlets, which spread into the surface soil layers. Such a structure of the root system later on facilitates further transplanting of the seedlings from the beds into the field.

The method of starting crop plants by means of transplanting of seedlings is very ancient and is chiefly applied in severe climates. The seedlings are usually raised in hotbeds or greenhouses during the colder season early in the spring, when there is sufficient light for successful nutrition and growth of plants, but the temperature in the open is too low. With the beginning of the warmer season, the seedlings are transplanted into the field. This allows a considerable increase in the length of the vegetative period; and by this procedure, such southern crops

as cucumbers and tomatoes can be grown in northern latitudes. For successful rooting of the seedlings in the relatively severe conditions of the open field, it is very important to have the root system concentrated in a small volume. For this purpose, the seedlings are not only transplanted, but the roots may be pruned.

In the practice of agronomy, the conviction is widespread that transplanting not only allows an extension of the length of the vegetative period, but that it accelerates growth and increases the yield, owing to a special stimulation. Based on this conviction, suggestions have been made to transplant into the field such crops as cereals, and special transplanting machines have been designed for this purpose.

At one time, this practice was much used in Germany. Trials of this method in practical agriculture did not confirm the hopes of its adherents. A more vigorous development of the individual plants after transplanting found its explanation in the fact that in applying this method the plants were placed much wider apart than was the case when the usual method of sowing the seeds in rows was followed. In calculating the yield per unit area, it was always found that the transplanted crop did not give a higher yield than drilled seeding and that the great expense for labor and costly machines was not justified. Exact physiological experiments performed by Krassovsky have shown also that transplanting causes a temporary check in growth and an expenditure of reserve substances for the restoration of the roots lost. Hence, this method is always connected with a certain increase in the length of the vegetative period and with a decrease in yield. It is of value only in cases when because of early cold or drought the plants cannot finish their development in time to escape unfavorable conditions and when it is important to begin their cultivation in a small space protected by glass while the weather is still cold.

92. Artificial Propagation of Plants by Cuttings and Layering. Regeneration and Polarity. Root-forming Hormones. Rhizocaline.—Because of a high degree of physiological independence that is characteristic of almost all parts of a plant and even of its separate cells, the latter have to a considerable extent the capacity for restoring all the lacking organs and of producing a completely new individual. This capacity has been extensively exploited in agriculture, and particularly in horticulture. Since

ancient times, a large number of methods for the artificial propagation of plants have been in practical use. Artificial propagation comprises methods that do not require the use of any special organs of sexual or asexual reproduction formed by the plant itself but that utilize any convenient and suitable vegetative parts.

The most popular method of artificial propagation is by means of cuttings and layers. By a "cutting" is understood any part of either the stem, the root, or even the leaf of a plant, which, having been separated from the plant and placed in favorable conditions for its development, is capable of growing. If such a vegetative part is left organically connected with its maternal tissues, though only during the first stages of rooting and growth, then it is called a "layer." Both of these methods are very popular among fruitgrowers and gardeners. Layering is used when cuttings do not root readily, *e.g.*, in the case of grapes, hazelnuts, mulberries, gooseberries, etc. In order to obtain a layer, a branch is usually bent down and covered with soil for some length, the tip being turned upward and left uncovered. The covered section in time will produce adventitious roots, after which the branch may be cut off and the layer separated from the maternal plant (Fig. 121). Sometimes rooting does not occur under this procedure. Then it is necessary either to make incisions on the part of the branch that is covered with earth or to girdle it. A callus is usually formed above the incision, or girdle, from which roots will arise.



FIG. 121.—Showing a method of obtaining a layer (*b*) from a grapevine (*after Molisch*).

Cuttings usually consist of sections of the stem, either with or without leaves. Their lower ends are placed in soil or sand to a certain depth. The soil used for this purpose must be very loose, preferably sand, and the cuttings have to be placed in an inclined position, so that their lower end is not too deep in the soil. This is necessary because the healing of the wound caused

by cutting and the subsequent formation of roots lead to an intense respiration; therefore, a liberal access of air is required. High humidity is another necessary factor for successful rooting. This prevents the cuttings from drying before they have had time to develop their own root system. This is essential for cuttings that bear leaves. Hence, while they are rooting, cuttings are often kept in greenhouses or in propagating beds under glass. Some cuttings, like those of willow, root very readily within a few days; while others, such as conifers or camellias, require several weeks or even months.

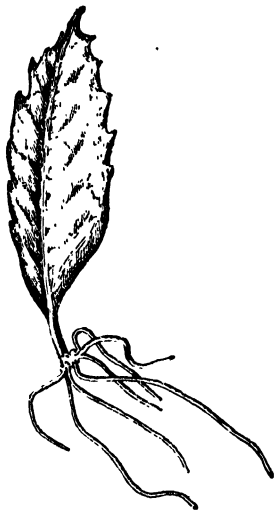


FIG. 122.—Rooted leaf of *Aucuba japonica* (*after Molisch*).

Cuttings also may be obtained from the roots of certain plants. These are placed in a somewhat inclined position and covered completely with light sandy soil. Adventitious buds will then be formed at their upper ends, which will produce shoots, while the roots will be formed from the lower end.

A leaf from certain plants may also be used as a cutting. It is planted with its peduncle in moist soil. With many leaves, this will result in the formation of roots on the surface of the cut, just as in the case of stem cuttings. On account of the absence of buds, many leaves produce no aerial organs and are incapable of developing into a new plant (Fig. 122). They may only increase in size somewhat but—what is of particular interest—will live much longer, even for several years; while, on the tree, they might have lived at the most only one or two years. There are a few plants whose leaves are able to form adventitious buds when they are separated from the mother plants. The best-known instance is the leaves of some of the Begonias. If one of their leaves is placed on moist sand and the larger veins are cut through, buds will appear at the points of incision and will later develop into new plants. This is a common gardener's method of propagating Begonias by means of leaves. Some varieties of *Cardamine*, *Bryophyllum* (Fig. 123), tomato, the

hyacinths, and other plants have also the capacity of producing buds from leaf cuttings.

Cuttings that are separated from the plant even when very small in size may regenerate all the other parts of a plant. Regeneration activity begins with the formation of callus on the surface of the cut tissues. This results from an increased division of cells adjoining the surface wound. Certain cells that had completed their growth long before the injury was performed may divide again under such conditions.

Especially rapid is the formation of the callus in the immediate vicinity of the phloem. This has given rise to the supposition that the phloem carries special hormones that stimulate cell division. In the case of injuries, the hormones enter the cells adjoining the phloem, inducing an increase of growth and division.

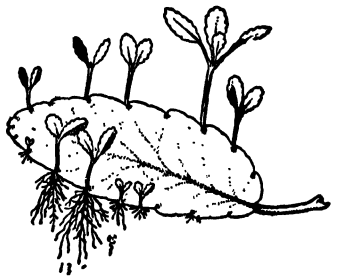


FIG. 123.—The formation of shoots on a *Bryophyllum* leaf (*after Transeau*).

The callus consists primarily of undifferentiated parenchyma cells in which the conducting elements, the cambium and regions of growth, are later differentiated. Numerous roots are developed also from the cambium of the cutting, but new shoots are formed with great difficulty from the callus. In order that propagation by cutting may be successful, it is important, therefore, that there should be on the cutting at least one bud capable of developing into a new shoot. In this connection, it should be noted that the so-called "dormant buds," which normally would not have developed for many years, if at all, will produce shoots on a cutting.

If a small section is cut from the middle of a branch having two injured surfaces, one at the top and the other at the bottom of the cutting, the callus at the morphologically lower end will develop much faster and more extensively than at the upper end. This cannot be attributed to a downward movement of organic substances, as might appear probable at first sight; for if such cutting is placed upside down, so that its morphological lower end will be at the top, the callus will still be more pronounced

at this end, though the force of gravity will act under these circumstances in the opposite direction (Fig. 124). When sections of axial organs react differently at their two morphologically opposite ends, the phenomenon is known as "polarity."

Polarity manifests itself clearly in the distribution of roots and the development of buds of a cutting. If a cutting is suspended in normal position in a humid atmosphere, the roots will be formed largely at its lower end, and the shoots will be produced from buds situated at its upper end. But when the shoot is suspended in an inverted position, the roots will nevertheless rise from the morphologically lower end, though it will now be at the top; while the shoots will develop at the morphologically upper end, now at the bottom. If the center of the cutting is girdled, the effect will be the same as that of the division of the cutting into two separate parts. This leads to the conclusion that polarity is probably caused by the accumulation of some substances that move in the bark in a definite direction and that stimulate at the point of their concentration the development of both the callus and the roots. Sachs has named these "rootforming substances." Some recent investigations, especially those by Loeb, have led to the conclusion that these are hormones. According to Loeb, the root-forming hormones originate in the leaves, whence they descend through the stem. As long as a branch is attached to the tree, no formation of roots takes place; but if the vessels through which the downflow of these hormones proceeds are cut, then the latter will accumulate at the surface of the cut and cause the formation of roots.

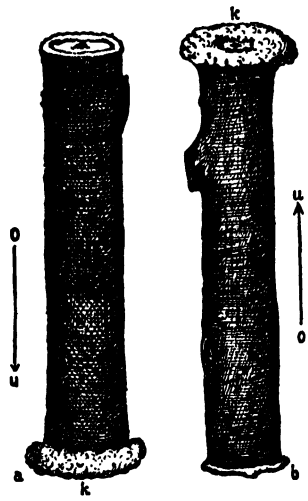


FIG. 124.—Poplar cuttings suspended in humid atmosphere. On the left, in normal position. On the right, in a reversed position, showing polarity in callus formation (*after Molisch*).

If a willow cutting is ringed, *i.e.*, if a ring of bark about 1 to 2 cm. wide is removed, and the cutting is then placed in water in such a way as to leave the ringed part in a moist atmosphere, the adventitious roots will develop much better and in greater

numbers above the ring cut (Fig. 125). On the control cutting, the greatest number of roots will develop at the extreme end. Of course, the impeding of the flow of nutritive substance by the ring cut plays a certain role, but a greater influence is exerted by the retention of the root hormone.

Recently the Belgian physiologist Bouillenne and the Dutch physiologist Went demonstrated quite definitely the existence of a special root-forming hormone, which they have termed "rhizocaline." Unlike the growth-promoting hormone, auxin, which was mentioned in Art. 29 and which is elaborated in the growing tip of the stem, rhizocaline is produced by leaves during photosynthesis. This explains the fact, long ago observed, that the remainder of at least one leaf on the cutting is of great significance for varieties that root with difficulty and that in such cases rooting proceeds more readily in the light. The base of the cutting, *i.e.*, the place where the roots are formed, must be shaded, however. Rooting is considerably stimulated by girdling or ringing of the cutting before its removal from the mother plant (Eremeev, 1933). This stops the outflow of rhizocaline and thus contributes to the initiation of roots above the girdle. After cutting and covering the base of the cutting with soil, these root initiations develop rapidly.

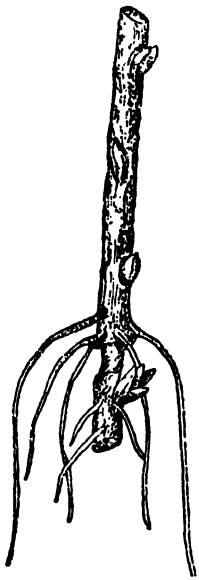


FIG. 125.—Girdled willow stem with roots growing primarily above the girdle.

93. Grafts and Chimeras.—If a cutting of a plant is not allowed to root but is set upon another plant, with which it is capable of uniting by growth, it will begin to develop and eventually produce a new plant. But the cutting under such conditions will not form its own root system. This operation is known as "grafting." The transplanted cutting is called the "scion"; and the plant with which it is united, the "stock." Grafting is a method of propagation common in horticulture, as the properties of improved varieties are not inherited through reproduction by seeds. Moreover, the cuttings of many fruit trees do not root readily. Seedlings obtained from the seeds of wild fruit trees commonly serve as stocks for this purpose. They are usually

cut off at the crown, and a small cutting of an improved variety is then grafted upon the remaining stump.

If the stock and the scion are of the same diameter, the oblique surface of the one is placed over a similar surface of the other with the purpose of joining them by growth. This method is called "whip grafting." If the scion is much thinner than the stock, then "cleft grafting" is resorted to. The stock is split in two; and the scion, cut to a sharp wedge, is inserted into the cut. To avoid drying out of the junction, it is sealed with graft-

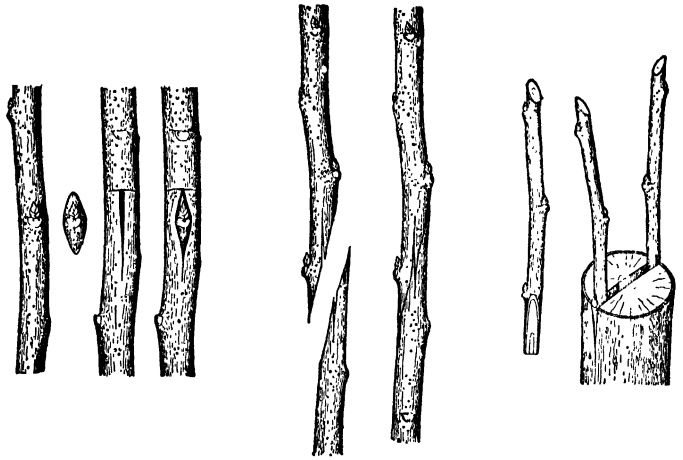


FIG. 126.—Methods of budding and grafting. Left, budding; center, whip grafting; right, cleft grafting (*after Brown.*)

ing wax and sometimes firmly bandaged. Instead of a whole cutting, very often only a bud with a small section of adjacent bark and wood is used for propagation purposes. This shieldlike cutting is inserted into a slit made in the bark of the stock. This method is known as "budding" (Fig. 126). Various other methods of grafting and budding are known, but these will not be discussed here.

With all the methods of this type of propagation, it is most important to ensure the closest contact between similar tissues of the same age of the stock and scion, as only such tissues can be joined together by growth. The callus plays the part of an intermediary agent in the process of subsequent differentiation of vessels and sieve tubes, which serve to connect the corresponding elements of both plants. In grafting, the contact of the

phloem elements is of especial importance. As has been seen, it is these elements that are the centers where the callus formation begins and where it is most vigorous. Very often a thickening of the stem, which is caused by an excessive development of the callus, is formed at the place of junction of the stock and scion. The joining of the two parts will be the easier, the nearer they are related to each other. One can graft different varieties of the same species, but it is also possible to unite two species of the same genus and even of different genera of the same family.

Various methods of grafting and budding have been used in horticultural practice since very ancient times. But only recently has the subject been put to a scientific analysis, chiefly through the investigations of Vöchting (1892), Winkler (1912), and Krenke (1928). The most important question seems to be the reciprocal effect of the stock and the scion, the two together representing a united organism. The roots of the stock supply the leaves of the scion with mineral nutrients, while the leaves, in their turn, send to the roots organic compounds. Still, despite this very close relationship, the two parts of a grafted plant are independent of each other, each preserving its own peculiarities as well as its specific storage products. Thus, for instance, in the sunflower, carbohydrates are stored in the form of starch; while in the Jerusalem artichoke (*Helianthus tuberosus*), the storage form is inulin. Now if a sunflower is grafted upon the artichoke, tubers of the usual type are formed on the roots of the latter, and these are filled with inulin, though the material for its production is supplied by the sunflower. Sometimes, however, specific qualities are transmitted from one part of the graft to the other. For example, in grafting tobacco upon the potato, the specific alkaloids of the tobacco are transported to the potato stock.

Still, in grafting, a quantitative effect is more common than is a qualitative one. In grafting an apple tree upon the paradise stock or a pear upon the quince, the time of fruiting is hastened, which often is of great advantage. The duration of life, however, is shortened. An apple tree upon paradise stock dies at the age of 20 to 25 years, while normally it may live for 200 years. While a reciprocal effect of the stock and the scion undoubtedly exists, it must be, generally, insignificant. A score or two of choice

varieties of apples may be grafted into the crown of a wild apple tree, and each of them will preserve all its specific properties and will produce fruit of a quality characteristic of the particular variety. Preservation of the characteristic of a variety is only possible because the reciprocal effects between the stock and scion are very insignificant.

"Chimeras," which at one time were called "graft hybrids," supply the most evident proof of the fact that each constituent of a graft preserves its own characteristics in spite of their close connection. They have been studied in detail by Winkler. By chimeras are meant plants that are sometimes obtained as a

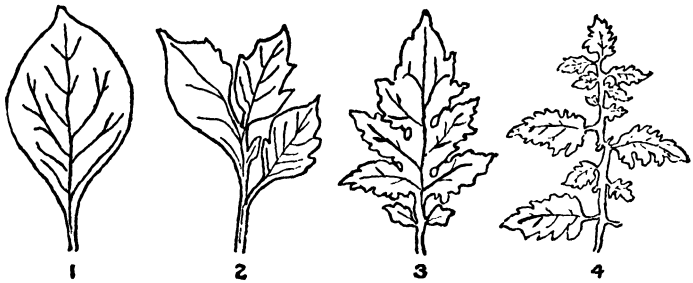


FIG. 127.—Black nightshade and tomato chimeras. 1, Leaf of a normal black nightshade; 2, leaf of an anticlinal chimera, 3, leaf of a periclinal chimera; 4, leaf of the tomato (*redrawn from Winkler*).

result of grafting one plant upon another and securing a combination of tissues of both. At first it was supposed that in these cases a fusion of cells had taken place, similar to that occurring in fertilization; hence the name, graft hybrids. Winkler's investigations, however, have shown that this is not so. He determined with accuracy both the nature of chimeras and the method of obtaining them. Chimeras are easily secured from various species of *Solanum*, especially *Solanum nigrum* and *Solanum lycopersicum*. The latter may be joined to the former by cleft grafting. A stump of the nightshade plant, *Solanum nigrum* is split, and into it is inserted a wedge-shaped tomato scion. They are then left to unite. When they have grown together, the scion is cut near the union. This results in the formation of adventitious buds on the cut surface. Those formed from the surface of the stump will develop shoots of *Solanum nigrum*, while those rising from the wedge will produce tomato shoots. Sometimes buds are formed at the point of junction of

the stock and scion, however, and then they will develop into shoots of a combined nature. These will be chimeras, in which one-half of the tissues will belong to one plant, the other half to the other. And if, for instance, the boundary line passes across a leaf, the latter will be of an asymmetrical form (Fig. 127, 2). Such chimeras are called "anticlinal."

Winkler has obtained chimeras of a still more peculiar nature. Sometimes the buds are so formed that the outward layers of the growing points belong to one plant and the inner tissues to the other. As a result, all the organs developing from these buds consist of two kinds of tissues, the external tissues of one kind and the internal of another. According to their external appearance, such chimeras, called "periclinal," show an intermediate character between the two species [Fig. 127 (3)], but cytological investigations have shown that the tissues of each component preserve the number of chromosomes characteristic of it, as well as other peculiarities. Hence, even in this close union, the reciprocal effect of the two species that are closely joined by growth is not very great.

Other types of chimeras have been obtained, and it has been established that some varieties that have been reproduced vegetatively for a long time, *e.g.*, *Cytisus Adami*, many varieties of *Pelargonium*, some clones of potato, etc., are chimeras with their inner tissues belonging to one plant and the exterior to another. While the fruits of such chimeras are usually of a mixed nature, the seeds always belong to the plant that forms the inner tissues. Chimeras can be preserved only by means of vegetative propagation.

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CHAPTER XII

PHYSIOLOGY OF THE DEVELOPMENT OF PLANTS

94. Normal Cycle of Development of Higher Plants. Annual and Perennial Plants. Interrelations between Growth and Development.—The life cycle of a higher plant, as well as of any organism, begins with the primary division of the fertilized egg cell and ends with the death of the individual. The early part of the life cycle is characterized by growth and the development of organs. This is succeeded by the stage of reproduction, which is followed by senescence and death.

The length of life of plants varies within broad limits. If only the higher plants are considered, there may be found ephemerals, like *Stellaria media*, which complete their development within a few weeks; and very large trees, some attaining an age of several hundred years, and others living several thousand years, like Sequoia in California. It should be emphasized here that there is a great difference between animals that have attained senility and plants in the same state. In an animal organism, almost all its tissues and organs are of the same age as the organism itself; while in a thousand-year-old tree, all its active parts, the leaves, buds, tips of shoots, and roots, serve only for a short time and are then superseded by new ones. For instance, the leaves of a century-old oak tree are only a few months old. Only the innermost layers of the trunk of the tree, which usually are dead, may have originated in the distant past.

As a rule, trees and other perennial plants have the capacity of rejuvenating annually. They therefore have no definite length of life. Some of their parts die each year and are again restored. The cells of the embryonic regions of a thousand-year-old tree are as young as those of a year-old seedling. At any rate, it has not yet been possible to discover in many the indisputable symptoms of aging. It is rather the definite relationship between the vegetative growth of a plant and its reproduction that is characteristic of its life cycle, and not so much the total duration of its life.

From this point of view, plants may be divided into two large groups: those fruiting only once, and those fruiting repeatedly. To the first group belong primarily the annuals, which complete their life cycle within one vegetative period. For the sake of convenience, the cycle of development will be considered not from the moment of embryo formation, but from the time of germination of the seed, the peculiar period of seed development thus being left out of consideration. The following are characteristic steps in the life of an annual. The seed germinates; the young plant rapidly develops its vegetative organs, and then it blooms; fruits are set and ripen; finally, all the organs of the plant begin to age rapidly and die; and the plant then dries up. As the period of drying frequently coincides with the autumnal season, it may seem that the death of such a plant is determined by external conditions. In reality, however, it is dependent upon internal causes. Many early ripening annuals, *e.g.*, the various early varieties of crop plants, produce seeds and dry up long before the end of the growing season. This behavior is still more marked in the ephemerals (Art. 85).

Blossoming and fruiting do not always occur during the first year. Often it is accomplished in the second year of the life of a plant. This is true of all biennial plants, such as carrots and beets. They live through the winter in a dormant condition, resuming growth only the following spring (Fig. 128). But fruiting of these plants is also followed by death. Finally there are some plants that develop for many years nothing but vegetative organs, attain a considerable size, and then begin to produce fruit heavily. In such cases, likewise, fruiting is followed by drying up of the plant. The agave, sometimes called the "century plant," belongs to this group. In Mexico, its native land, it will begin to blossom at the age of 8 or 10 years. Under less favorable conditions of development, as on the coast of the Mediterranean Sea or in greenhouses, a more protracted period, 20 to 50 years, is required for its development previous to blooming (Fig. 129). Some of the palm trees, among them the famous shade palm of Ceylon (*Corypha umbra culifera*), display the same characteristic behavior.

It has not been possible as yet to determine what inner processes cause the dying of plants subsequent to fruiting. But the fact that there exists a close causative connection between the

two has been proved by numerous experiments. The life of many plants may be considerably prolonged by removing their floral buds and thus preventing fruiting. By this method it is possible, for instance, as has been done by Molisch, to make an



FIG. 128.

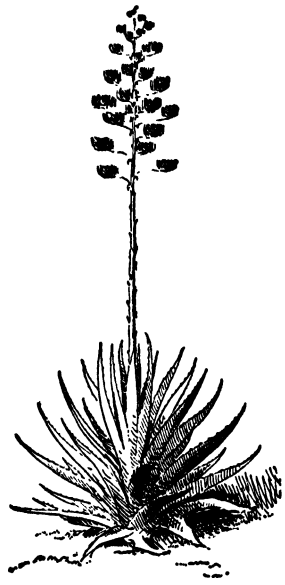


FIG. 129.

FIG. 128.—Stages in development of the wild carrot, a biennial plant. On the left the seedling; in center, plant at the end of first growing season, having formed a fleshy root. On the right, a mature and blooming specimen in the middle of the second year (*after Transeau*).

FIG. 129.—Century plant (*Agave*) showing rosette of fleshy leaves and flowering stalk (*after Transeau*).

ordinary garden mignonette live 2 or 3 years instead of 1 year. It will then acquire the form of a small shrub. On the other hand, when biennial plants, for some reason or other, produce seeds during the first year—the so-called "shooting to seed" in beets and other root crops—then their vegetative organs will die promptly.

Other types of plants are characterized by repeated blooming. Here belong the great majority of perennials and woody plants. They blossom and fruit every year. Still, that does not prevent their further vegetative growth. The duration of their life is practically unlimited. What inner physiological readjustments enable them to continue their vegetative growth after fruiting are at present unknown. In some plants like the tomato and cucurbits, alternate cycles of growth and fruiting may follow each other in successive order. The contents and requirements of the fruit seem to control growth in such plants (Murneek).

Although the accomplishment of its life cycle is usually accompanied by the growth of the plant, nevertheless these two phenomena are far from being identical. Growth basically consists in an increase in size and the repeated formation of vegetative organs, stems, leaves, and roots. The chief function of these organs is participation in the further accumulation of organic substances of the plant. The most important symptom of the development of a plant, its transition to sexual reproduction, must be regarded as the origin of a new quality, *viz.*, the ability of the plant to reproduce by seed, occurring as a result of a series of quantitative changes. As has been shown by the investigations of Lysenko, the transition of the plant to the reproductive phase occurs not suddenly but gradually by a sequence of stages of transition through definite stages of development, terminating in the formation of ripe seed similar, but not identical, to the seed from which the plant was originally developed. By sexual propagation alone, the individual dies, but the species continues to exist and to develop in the alternation of generations.

95. Conditions for the Formation of Reproductive Organs. Hypothesis of Klebs of the Significance of Changes in the Chemical Composition of Plants.—Definite conditions must be realized for the transition of a plant from the vegetative to the reproductive phase. Without these conditions, the transition to reproduction is impossible, and the plant remains sterile during an indefinite period. The understanding of these conditions is of great theoretical and practical importance; for if these conditions are known, the development of the plant may be influenced in the desirable direction, *i.e.*, the cycle of the development may be controlled by changing the conditions. As has been seen, such a control represents one of the chief problems of plant physiology.

For a long time, it was supposed that the cycle of development is determined exclusively by internal hereditary periodicity and that this cycle or rhythm of development being predetermined is strictly constant and does not change readily under the influence of external agencies. The age of the plant was assumed to be the chief factor determining the transition to reproduction. Thus, some varieties are precocious and mature much earlier than other later varieties. Among the trees and shrubs, there are species that bloom at the age of 5 to 10 years, as for instance many fruit trees; those like the maple, the linden, and the larch that flower at the age of 25 to 30 years; and finally, those like the oak, the elm, and the chestnut that are mature only at the age of 40 to 60 years. Though in horticulture wide application is being made of methods of accelerating the time of fruiting, chiefly by means of grafting on dwarfing stocks, nevertheless up to the present there have been no serious attempts at systematically elaborating methods of controlling the length of the vegetative period.

Klebs was the pioneer in the field of the physiology of plant development. In his investigations, which were carried out at the end of the nineteenth and at the beginning of the twentieth century, Klebs was the first to affirm that the cycle of development of the plant can be changed at will by the interference of man. According to his conception, the development of the plant, as well as its other vital processes, are greatly influenced by environmental conditions; and if these conditions are changed, development can be changed, retarded or accelerated. Alterations in environmental conditions act upon the processes of reproduction not directly but through changes brought about in the internal state of the plant.

According to Klebs, one of the most important factors contributing to the transition to reproduction is a sufficient light intensity. If a plant is grown under conditions favorable in all respects except for an insufficiency of illumination, blooming will be considerably delayed and sometimes does not occur at all. Klebs, for instance, grew, in diffused light, specimens of *Glechoma hederacea*, which remained in a vegetative condition for several years. But when the plants were placed in a stronger light, they soon began to bloom. Such experiments have been successful with other plants, also.

This effect of light, however, is more of an indirect than of a direct nature. Plants that have a considerable storage reserve of organic substances, especially carbohydrates, will bloom even in darkness. The hyacinth and other bulbous plants are good examples of such behavior. Similarly, it is possible to obtain flowers on separate branches of a plant, which are placed for this purpose in a dark chamber, provided that their organic connection with other parts of the plant remains intact and the rest of the plant is exposed to strong light. On the basis of these facts, Klebs was led to the idea that light hastens the period of blooming chiefly by assisting the plant to accumulate carbohydrates and that the latter is the direct cause of the change from the stage of vegetative growth to that of reproduction.

This has been confirmed by experiments with plants grown under different kinds of colored glass, each representing a certain part of the spectrum. In red light, plants bloom almost as readily as in white light; while blue light delays blooming, despite the fact that the formative effect of light depends chiefly on exposure to blue rays. Analyses have shown that plants grown in red light are much richer in carbohydrates, both starch and sugars, than those grown in blue light.

Besides the carbohydrates, the amount of mineral substances and various nitrogen compounds also is of importance in determining the time of fruiting. It has been noted for a long time that nitrogenous fertilizers increase the vegetative growth of a plant at the expense of its reproductive functions. According to Klebs, it is not the absolute amount of particular substances that determines the time of fruiting, but their proportional relation. A preponderance of carbohydrates over nitrogen and mineral compounds leads to blooming and fruiting; a reverse relationship, to vegetative development. Hence, by combining certain fertilizers and by other cultural methods, one may produce in the plant either an accumulation of carbohydrates or an increase in nitrogenous compounds, thus making it possible to control to a certain extent the development and behavior of plants (Kraus and Kraybill).

A number of methods used by fruitgrowers, for the purpose either of accelerating blooming or making fruit production more abundant, are based also on the relationship between carbohydrates and soil nutrients. Transplanting, the cutting of roots,

or a temporary neglect of soil tillage are a few of such practices. These procedures, however, have the disadvantage of curtailing the general development of a plant. More reasonable are the methods that serve to check the removal of assimilates from the stem or the individual branches. This may be accomplished by girdling the bark or subjecting it to pressure by means of a wire or by partial twisting or breaking of the branches. Probably the most effective method of increasing fruitfulness is the use of the tree girdle, which is popular in Germany (Fig. 130). This girdle consists of a thin strip of zinc, which is firmly tied around the bark of the stem by means of wire, thus producing pressure and interfering with the downward flow of organic substances. The swelling formed above such a girdle is an evidence of this fact. A belt of this type does no permanent injury to the bark. It can be removed when not wanted. The general increase in fruitfulness due to grafting should be attributed, at least partly, to an interference with the downward flow of organic substances at the graft union.

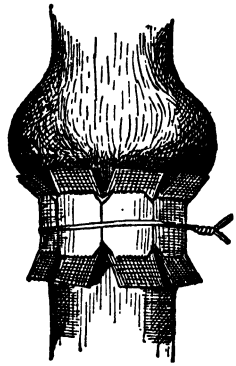


FIG. 130.—Tree girdle on a lilac branch. Note callus above the girdle (*after Molisch*).

The water relations in plants also have a certain effect on the determination of the time of fruiting. A dry air usually favors fruiting, while high humidity delays or suppresses it entirely. A dry soil, on the other hand, appears to be less favorable to fruiting than a humid one. Consequently, the conditions that are most favorable to fruiting are those attained on well-irrigated land in an arid climate with a large number of bright days, as for instance in California or Central Asia. Under such conditions, fruit trees generally yield very large crops.

Klebs's theory of the significance of the relationship between carbohydrates and nitrogenous substances has become popular in America. By studying the question of fruiting of tomatoes, Kraus and Kraybill (1918) have contributed much to the establishment of the significance of this concept. During the last 10 to 15 years, it has been shown that other proportional ratios of nutrients are just as important as the C/N ratio alone. Although it exerts a certain influence on the abundance of fruiting, which

is also dependent upon the conditions of nutrition, nevertheless the time of the transition to reproductive development is not determined by the accumulation of one nutritive substance but may be dependent upon specific factors, probably flower-producing catalysts (Murneek).

96. Theory of Lysenko on the Stages in Development of Plants. Yarovisation, Its Theoretical Foundation and Practical Significance.—The most important advance in the physiology of development is the method of shortening the vegetative period elaborated by Lysenko, which has become widely known as "yarovisation."

The method of yarovisation of plants is very simple and convenient. It is based on the principle that seeds just beginning to germinate are subjected to definite external influences that accelerate certain physiological processes causing a transition to the reproductive stage. The earliest case of yarovisation that has been studied is the transformation of winter into spring crops, or rather the stimulation of heading of winter crops during the same season that they were sown, similar to spring crops. In this case, the leading factor of yarovisation is lowering the temperature during a sufficiently prolonged period.

According to Lysenko, winter crops sown in spring do not fruit because at a definite period of their development they require a low temperature for 20 to 50 days, depending on the variety. The temperature must be below 10°C., but 2 to 0°C. is preferable. Winter crops sown in the field in autumn are subjected under natural conditions to the influence of the temperatures necessary for the process of yarovisation. After they have remained long enough at low temperatures, they do not require any more cold exposure for their further development. When sown in spring, the winter crops do not obtain the cold exposure that is indispensable for their transition to reproduction and are therefore incapable of heading. When sown in spring, they remain vegetative throughout the summer, tillering profusely and producing continuously new leaves but no culms. If they are yarovised artificially, *i.e.*, if during germination they are kept cold for a sufficiently long period, they will pass through the stage of development that requires a lowered temperature and will head without retardation even when sown in spring (Fig. 131).



FIG. 131.—Spring-sown winter wheat. *A*. Plant sown without any treatment has not headed. *B*. Plant from yarovised seeds has produced heads normally.

Lysenko's method of artificial yarovisation is based on the interesting feature that the plants are subjected to the influence of the complex of external factors indispensable for the transition to reproduction in their earliest germination period, and not in a mature state when it is difficult to reproduce the necessary complex even under laboratory conditions, not to mention in the field, where it is almost impossible. In order to retard growth during the comparatively prolonged period of yarovisation, 20 to 50 days, the seeds are given only 50 per cent of the total amount of water necessary for germination. With such a degree of moistening, most of the biochemical processes obtaining in the cell during the active state continue their course, while growth is considerably retarded. Such a curtailment of growth is necessary for the sake of convenience in the transport and sowing of yarovised seeds; for otherwise the seedlings with elongated rootlets and plumules might be injured.

Lysenko bases his method of yarovisation on the following theoretical concepts:

1. Growth and development are not identical phenomena.
2. The development of an annual seed plant consists of separate stages.
3. The stages always proceed in strict sequence, and the successive stage cannot begin so long as the preceding stage has not terminated.
4. The different stages of development of the same plant require various combinations of external conditions.

Lysenko lays greatest emphasis upon the idea that growth and development are two different phenomena. Growth is the increase in size of a plant without deep qualitative changes of the growing parts. Development according to him is the alteration of the separate stages that advance the plant toward reproduction. The transition from one stage to the other is accompanied by profound qualitative changes, but these may not be accompanied by any apparent external morphological evidence.

Hence, the physiological stages of development differ sharply from the external well-marked phases of development, which are commonly noted in phenological observations, when the vegetation of a plant is described. Such phases as the appearance of the seedlings, the opening of the first and second leaf, tillering, shooting, heading—all these denote the separate phases of growth independently of the stages of development. For

instance, winter plants not yarovisated may tiller profusely, form numerous leaves, accumulate large amounts of organic substances, and at the same time remain in the same vegetative stage of development. Transition to the following stage will be possible only after the plants have remained for a sufficiently long time under conditions favorable to yarovisation, *i.e.*, after they have been subjected to the influence of low temperatures with a sufficient access of oxygen.

Not all of the internal stages of development have been sufficiently established as yet. Lysenko describes two: the *stage of yarovisation*, requiring the conditions that have already been stated above; and the *light stage*, directly following the preceding one. Winter cereals require for passing the light stage a prolonged diurnal period of illumination, no less than 11 to 12 hr., but the best results are obtained with continuous illumination. Low temperature is no longer required. On the contrary, the light stage is accelerated by higher temperatures. But if, after yarovisation, the plants are given an insufficient day length, for instance 10 hr. or less per day, they will not form heads but will continue tillering, like plants that have not been yarovised.

Conditions necessary for the transition from one stage to another may be different for plants of various physiological types. In yarovising the seeds of millet, for instance, they must be subjected during 5 to 7 days to a temperature of about 20 to 25°F., and their light stage proceeds best in a short day or, still better, in complete darkness (Fig. 132). Similar conditions are required for the yarovisation of corn, cotton, and other thermophilous plants of southern latitudes.

According to Lysenko's theory, each stage of development is accompanied by qualitative irreversible changes, which occur in the protoplasm of the plant cells, chiefly in the meristematic region. What exactly these changes are, has not yet been established with certainty. It has been observed that during the stage of yarovisation changes occur in the oxidation-reduction potential in the meristematic cells (Richter, 1933). Some authors assume that specific hormones of reproduction are accumulated similar to the sex hormones of animals.

The theory of yarovisation has not been developed completely as yet. Lysenko, as well as other investigators, is actively

engaged in working out its details, and it is very probable that sooner or later it will undergo certain changes. But as it is, it represents a great step forward on the way toward ascertaining the laws of the development of plants and provides useful methods for controlling development in the desired direction.

The practical application of the methods of yarovisation is extremely diverse. Shortening the vegetative period in cereal

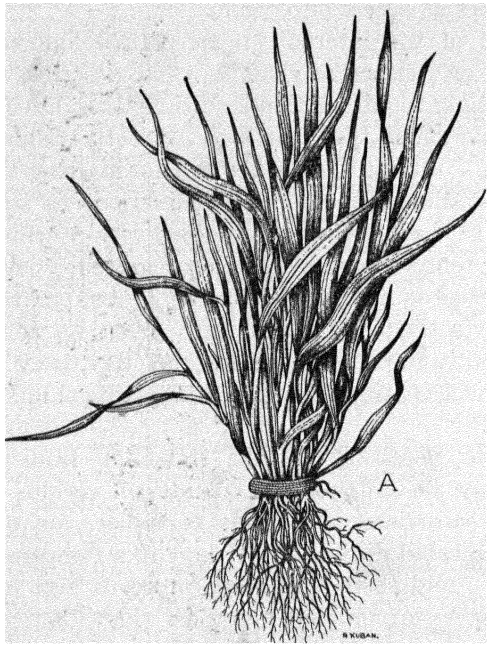


FIG. 132.—Millet plants from non-yarovised seed, *A*, and plants from yarovised seed, *B*, of same age.

crops is one of the most important means of escaping drought, especially in regions where drought occurs chiefly in the latter part of the summer, as in the north central Great Plains and in South and Southeast Russia, where even in relatively moist years the soil is completely dried towards autumn. In such regions, it is possible now to sow valuable late-wheat varieties. Thus, the vegetative period is shortened by means of yarovisation, and they escape the late drought; while before the elaboration of the method of yarovisation, it was only the less valuable early varieties that could be sown in these regions.

Yarovisation likewise is of great significance in the northern latitudes with a short summer, for it allows the cultivation in these regions of important late varieties of wheat, oats, and other crop plants. Yarovisation of millet, cotton, corn, and other thermophilous plants is also of great importance in allowing the culture of these in more northern latitudes.

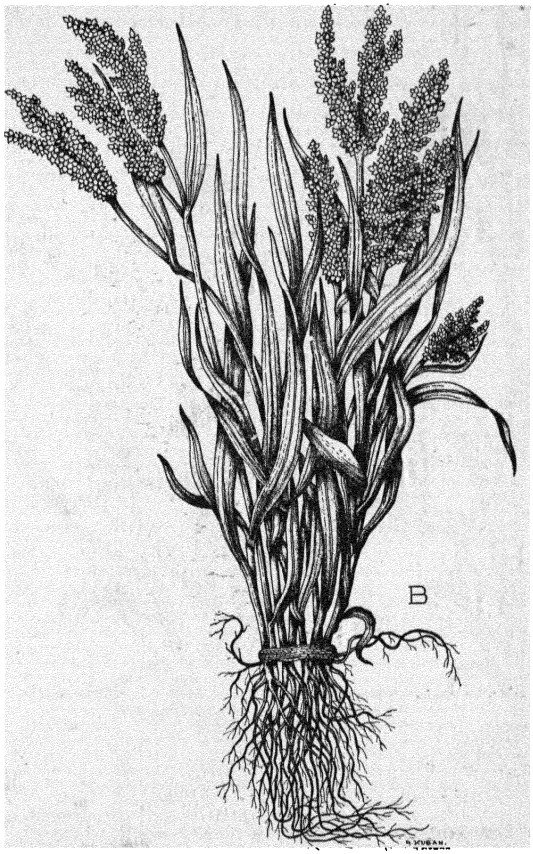


FIG. 132*B*.

The practical significance of yarovisation is increased by the fact that this method not only accelerates the development of crop plants but also increases their yield.

The influence of yarovisation appears very early in the plants. The seedlings from yarovised seeds grow more rapidly and more uniformly, this by itself being of great advantage. An increase

in yield of yarovised plants may be observed not only in especially cold or droughty regions but everywhere. Mass experiments in yarovisation carried out all over Russia in the collective farms in 1934 gave an average increase of yarovised wheat as

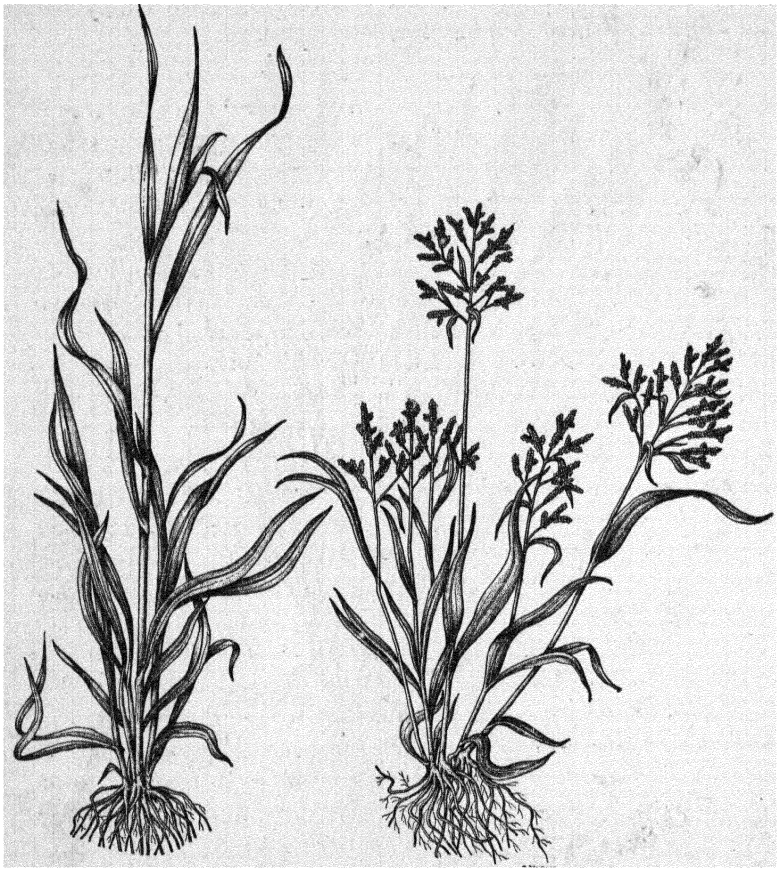


FIG. 133.—Development of short-day plants—millet. On the right, plants grown in 9 to 12 hr. of daylight per day. Left, plants grown in 18 hr. daylight per day. Both sets are the same number of days old.

compared with those not yarovised of about 1.13 cwt. per hectare; in some cases, the increase attained 2 to 3 and even as high as 9 cwt.

97. Influence of Length of Day on the Development of Plants. Photoperiodism.—Garner and Allard's investigations, which have

been confirmed by a number of others, showed that in determining the time of blooming very great importance must be attributed to the relative length of day and night. Some plants, *e.g.*, many varieties of soybeans, cotton, beans, asters, and cereals such as millet, sorghum, and rice, bloom earlier, the shorter the days and the longer the nights are. Consequently, under natural conditions these plants will produce flowers only in autumn when the days become shorter. By an artificial decrease of the day, as for instance, if the plants are placed in a dark room during part of the day or are covered with opaque cloth, they may be made to bloom at the beginning of summer. Such plants have been called "short-day" plants (Fig. 133). They mostly are native to southern latitudes where the summer day is comparatively short. There are, however, many plants, *e.g.*, wheat, oats, and other cereals, that will bloom earlier, the longer the day and the shorter the night. By illuminating them with electric light during a part of the night or all night long, it is possible to make them bloom much earlier. These are called "long-day" plants (Fig. 134). Plants of this type have a shorter vegetative period in the northern latitudes, where the days in summer are very long and where the length of day overbalances the retarding effect of low temperature. These plants show the highest rate of development under conditions of continuous illumination.

The response of plants to the duration of night and day is known as "photoperiodism." It is of interest to note here that the nature of the response of plants to the photoperiod is to a considerable degree dependent on the origin of the plant. In the majority of cases, tropical plants are short-day plants, while those of the temperate zone belong to the group of plants requiring a long day. Hence, when transported to the tropics many plants of temperate zones will never bloom.

The photoperiodic reaction of different varieties of one and the same crop is not always similar. Thus, for instance, the soybeans, as has been indicated, belong to short-day plants. But some of the varieties, *e.g.*, the Biloxi, are considerably retarded in their development by an elongation of the day. Thus with a 16-hr. day as compared with a 12-hr. day, their vegetative period doubles. Other varieties, *e.g.*, the Mandarin, show little response to such a change and bloom simultaneously.

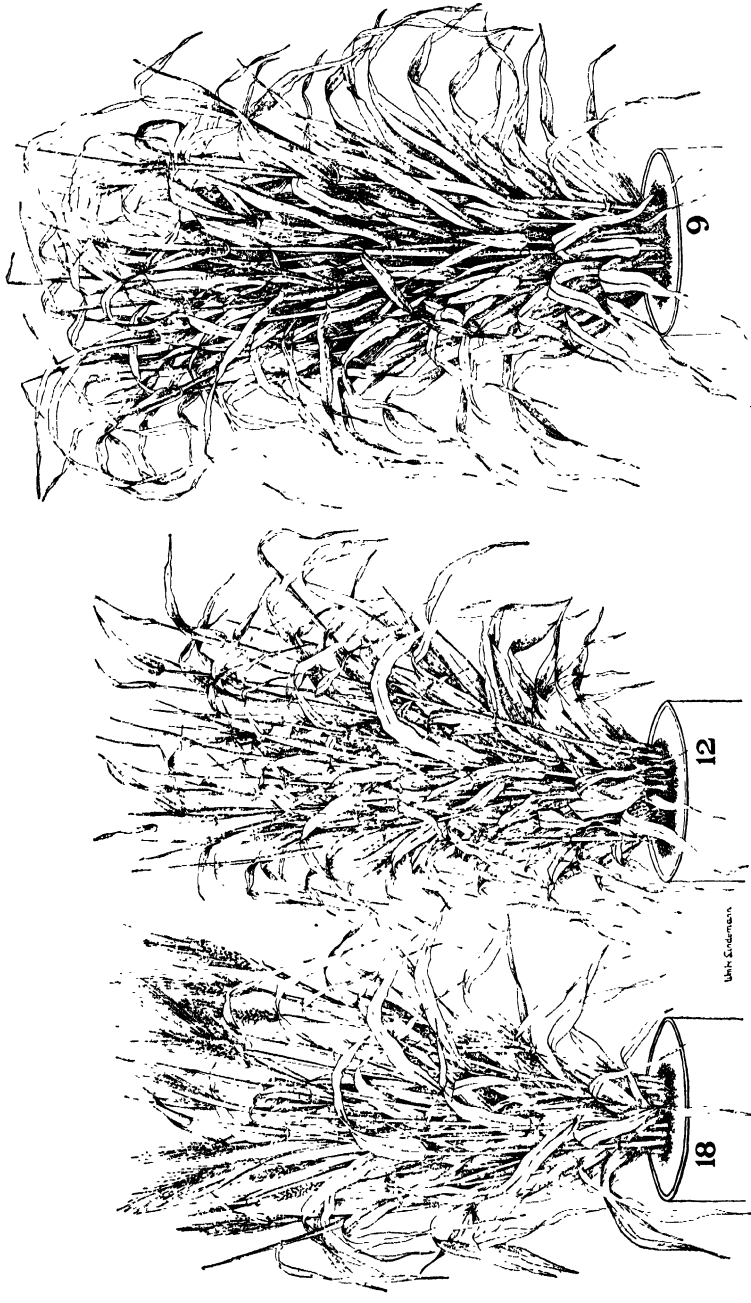


FIG. 134.—Development of a long-day plant, barley, in the same number of days under 18-, 12-, and 9-hour day.

The same differences may be observed with long-day plants, for example, with wheat. This proves that these differences are related to the geographical origin of the varieties. Northern wheats prolong very significantly their development when cultivated in southern latitudes with a shorter day, while certain other wheat varieties are indifferent to a shortening of the day.

The phenomenon of photoperiodism is of great significance in the distribution of varieties of crop plants in different localities and in moving them from one region to another. Long-day plants such as wheat are not hindered in their development when moved to the north, for the prolonged day of northern latitudes does not impede, but accelerates, their development. This makes the culture of such plants easier in northern latitudes and to a considerable degree compensates for the influence of the short northern summer. It is more difficult to move the short-day plants toward the north; for not only is the summer shorter there, but the long day increases their vegetative period. In such crops, varieties must be chosen for the northern latitudes that react as little as possible to an increase in the length of the day, *i.e.*, that have a lower photoperiodic response. Such knowledge of the photoperiodic reaction of separate varieties is very important.

The length of day is important not only in determining the time of blooming but it has a marked effect also on the development of vegetative organs. The curtailment of vegetative growth by the photoperiod is known as "photoperiodic inhibition" (Murneek). Short-day plants, for instance, will develop a much larger leaf surface and in general have more vigorous vegetative organs if the day is prolonged. When cultivated in northern latitudes, they do not mature; but since they produce a large mass of leaves, they may be used for hay and silage. This vigorous development of the leaves is a result of the fact that when blooming is retarded, the organic substances elaborated are directed toward the formation of new leaves, though in many cases photoperiodic inhibition is brought about directly by the length of day (Murneek). For the same reason, long-day plants develop a larger leaf surface and a larger total green weight with a short day, although the shortening of the day by decreasing the number of hours for the photosynthesis should reduce the accumulation of organic substances. With an excessively short day, *e.g.*,

6 hr. and less, a depression of growth may be observed. The relationship between the total size of plants grown at different lengths of day is clearly demonstrated in Figs. 133 and 134.

A very profound influence is exerted by the length of day on the development of tubers, bulbs, and other underground storage organs. In many plants, *e.g.*, in the garden onion, a long day promotes the formation of underground organs, the bulbs; while a short day on the contrary stimulates the formation of leaves. Many tropical plants, as for instance wild species of potatoes, are characterized in their native habitat in the equatorial regions of South America by an abundant tuber formation. They produce no tubers at all when grown in Central Europe with its longer summer day. But it is sufficient to shorten the day artificially to 12 hr. to obtain numerous tubers. In the cultivated potato, however, a long day does not impede tuber formation, and it gives excellent yields even beyond the Arctic Circle, where the sun does not set in midsummer. Here the influence of the soil temperature is most important. Potatoes native to high altitudes develop best at low temperatures, and an elevation of the soil temperature 22 to 25 per cent suppresses tuberization.

It is not necessary to subject the plant to a photoperiodic influence during the whole time of its vegetation. It is sufficient to give it the corresponding photoperiod during only its first period of development, from 10 to 15 or 20 days, to obtain the photoperiodic effect. Even when transferred to the reverse light conditions, it continues to develop in accordance with the photoperiodic effect of the first period (Garner and Allard, Egiz, Rasumov). This phenomenon has been termed the "photoperiodic after-effect," or "photoperiodic induction." Besides its great theoretical significance, this phenomenon has an important bearing on agricultural practice because it allows the limiting of the time of action of the light period, which may be more easily accomplished in practice with plants grown in hotbeds.

The relative length of day and night exerts a considerable influence upon the growth of trees. Those of different geographical origin react differently. Southern species are retarded in their growth by the influence of the long northern days. The shoots attain under these conditions a considerably larger size

but do not mature in time and are therefore injured by winter frosts. In species of northern origin, the ripening of the shoots is normal with a long exposure. The effect of the length of day upon the inception of dormancy will be discussed in detail in Art. 103.

Photoperiodic experiments are rather simple. They are based on the practice of covering the plants in morning and evening hours by opaque cloth or paper, thus decreasing the day length and increasing the night. Larger plants are covered with plywood covers mounted on wheels. For crops grown in pots, it is convenient to use trucks, which can be pushed in the evening into a dark house and in the morning back into the open. Opaque cloth covers are used in the United States to cover beds of aster, chrysanthemum, etc., grown outdoors or in greenhouses.

The nature of the photoperiodic effect is as yet unknown. Almost certainly it is not connected directly with the process of photosynthesis. This is proved by the fact that a very weak light, 5 to 10 candle-meters, is sufficient for the production of the photoperiodic effect. At such light intensity below the compensation point, photosynthesis is less than respiration. The necessity of excluding the morning and evening hours, *i.e.*, of prolonging the night, also confirms the statement mentioned above. Placing the plants in a dark chamber for several hours in the middle of the day only retards the accumulation of organic substance but does not exert any influence upon the time of blooming.

According to Lysenko, photoperiodism is the manifestation of definite light and darkness requirements of the plants during the second stage of development, designated by this author as the "photo stage" and following directly the temperature, or yarovisation, stage. For transition to reproduction, some plants require at this stage a definite period of complete darkness; others, a certain length of day. The first may be called really "long-night plants"; for the longer the night is, the sooner these plants will obtain the required number of hours of darkness. The second group of plants, which require during their light stage, not darkness, but a definite length of day, belong to the so-called "long-day plants." As this effect of light and darkness is not connected with photosynthesis, the light stage may be passed through at an early age even in germinating seeds that

are retarded in their growth, at least in the case of short-day plants. Seeds that have already passed through the light stage, when afterward sown under conditions of a long day, very soon bloom at any day length, even when given uninterrupted illumination. Unfortunately as yet it has not been possible to make all plants pass through the light stage in the form of germinating seeds, and this greatly limits the practical application of photoperiodism. The increase of the length of day by means of electric light is very limited in application being useful chiefly for greenhouse crops.

98. Physiological Differences between Winter and Spring Types, Early and Late Plants. Basis of Selection for Earliness. The discovery of yarovisation and of photoperiodism has changed completely the concept of early- and late-ripening plants. Before these discoveries, it was usually assumed that the length of the vegetative period of one variety or the other is the result of the inherent speed of its development, independent of the environmental conditions. It was known that the rate of development depends upon the temperature and that in hotter climates development is more rapid than in colder regions; but the sequence of earliness of the varieties was assumed to be constant.

At present, questions of earliness are approached in a quite different way. In the discussion of photoperiodism, it has already been emphasized that different varieties react differently to the length of day. Let us examine, for instance, the Mandarin and Biloxi varieties of soybeans, both typical short-day plants. With comparatively short day, both varieties mature simultaneously, but Biloxi is more susceptible to an increase in day length. When grown in the north, it is a later variety than Mandarin. In more southern regions, on the contrary, Biloxi will develop in advance of the Mandarin variety. Thus, earliness is only relative, depending upon the character of the reaction of varieties to changes in the environmental conditions. It must be noted that different phases of development depend to various degrees upon the environmental conditions. The length of day influences chiefly the duration of the period between the seedling stage and blooming, while the length of the period between flowering and ripening of the seeds depends chiefly on the temperature. That is why varieties blooming early but ripening slowly will react differently to changes in environmental

conditions when compared with varieties that are slow to bloom but ripen rapidly.

When plants are placed in conditions of day length under which they cannot bloom at all, *e.g.*, when either short-day plants are cultivated beyond the Arctic Circle, or long-day plants in the tropics, they completely lose their capacity to flower and continue vegetative growth for an indefinite period. They can bloom only when the day reaches its proper length. For short-day plants in the north, this will be in autumn. This leads to the phenomenon, first observed by Garner and Allard, *viz.*, that several varieties of soybeans, especially sensitive to the length of the day, bloom always at the same time of the year during the same autumn month, independent of the time of sowing. The length of their vegetative period is longer, the earlier they are sown.

The peculiarities of the stage of yarovisation are of no less importance in determining the length of the vegetative period. They are especially noticeable when spring and winter varieties are compared. Spring varieties mature the first summer, while winter varieties are sown in autumn and thus their vegetative period is considerably longer. But if winter crops are yarovised, *i.e.*, are made to pass through the stage of yarovisation in the form of seeds just beginning to germinate, they will head the first summer and frequently even earlier than many spring varieties. Hence, winter and spring varieties differ not in the length of their vegetative period but in their different temperature requirements during the stage of yarovisation. Winter crops need for the passing of this stage a prolonged period at a temperature from 0 to 5°C., while spring crops can do without it. Depending upon the time required for passing the stage of yarovisation, different degrees of "winterness" may be distinguished, according to Lysenko. Hence, the difference between winter and spring crops is not of a qualitative but only of a quantitative nature, and both groups are connected by a series of transitional forms.

The method of rapid determination of winter and spring seeds that has recently obtained wide use in the practice of seed control stations is also based on the different reaction of varieties to temperature. If unknown seeds are sown in a warm chamber and are further grown under continuous illumination, spring

varieties will head very soon, stimulated by the high temperature and long day, while the winter crops, which under such conditions cannot pass the stage of yarovisation, will remain for an indefinitely long period in the tillering phase (Maximov).

Earliness is thus the result of qualitatively distinct elements, and in breeding plants in this direction it is indispensable to account for all of these elements separately. In crossing varieties according to Lysenko's suggestion, one must not simply take two varieties that have proved early ripening under the given conditions, but the pairs must be so chosen that in one of the parents earliness should be determined, for instance, by a rapid stage of yarovisation, in the other, by a short light stage. In such a way, extremely early varieties may be obtained from two parents, each of which may be a late variety under normal conditions. Systematic selection and breeding of varieties with a definite length of vegetative period are not possible without a previous analysis of the conditions required for these two most important stages of development. Besides, it must always be remembered that varieties early under some conditions may be late under others.

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CHAPTER XIII

PHYSIOLOGICAL PROCESSES DURING FLOWERING AND RIPENING OF FRUITS AND SEEDS

99. Physiological Processes during Blooming. Respiration of Flowers. Nyctinastic and Seismonastic Movements.—It is known from studies of plant morphology that flowers represent modified shoots adapted to the functions of reproduction. The external parts of the flower, chiefly the calyx and in a lesser degree the corolla, representing leaves that are very little modified, participate in photosynthesis in their early stages of development owing to the presence of green plastids. From the time of the opening of the flower, this function becomes subordinate, and respiration becomes intensive. Flowers that are opening have been used like germinating seeds for demonstrating the respiration of plants. In massive flowers such as those of the aroids or of the gigantic water lily *Victoria regia*, a considerable increase in temperature takes place because of rapid oxidation. Such intensive respiration is accompanied by a considerable consumption of organic substances. Many authors who have studied the accumulation of dry substance of plants during their vegetative period have noted a marked decrease in the total weight of the plants during blooming, which may be due to cessation of vegetative growth at this time, to decreased photosynthesis, or to both. In perennial grasses and in woody plants, blooming usually occurs in the spring and is accompanied by utilization of the reserves stored during the previous summer. Annual plants also show a considerable depletion of food reserves of the stems and leaves at the time of blooming. This depletion usually reaches its maximum at the time of ripening of the fruit and seeds. Hence, in utilizing natural and cultivated meadows for hay, the grass must be cut at a time not later than the beginning of blooming of the grass varieties that predominate in the meadows; otherwise there will be considerable losses in the total amount of nutritive substances.

The rapid growth occurring in opening flowers is manifested not only in the general increase in size of the separate parts of the flower but also in the peculiar growth movements generally defined by the term "nastic movements," or "nasties." In Chap. IV, one type of growth movements, *viz.*, tropisms, has already been discussed. As has been seen, these movements are caused by the influence of some unilateral external factor, *e.g.*, light or gravity, acting in a definite direction. The growing organs are curved in a definite direction in relation to this factor. For instance, the tops of stems placed horizontally curve upward, or growing seedlings curve in the direction of light.

The term nasties is applied to such movements as are produced by stimuli that have no definite direction, resulting in no particular orientation of the plant. A dorsiventral or bilaterally symmetrical structure of the curving organ is indispensable for movements of a nastic nature. Those involving changes in turgor, otherwise called "alternating movements," are of far greater importance than in tropisms.

Nyctinastic movements caused by succession of night and day are very common. Many flowers open in the morning and close for the night. Some leaves, particularly the compound ones of papilionaceous plants, change their position during the night and day. These movements occur with considerable regularity, which led Linnaeus to attempt to construct a "floral clock" by making a bed of plants in which the different flowers would open and close at various hours of the morning and evening. Movements of this nature are often said to be connected with the "sleep of plants." This expression is inappropriate, as such nyctinastic movements have nothing in common with the sleep of animals, which is a peculiar condition of rest.

Changes in temperature or the intensity of light, or both, are the actuating causes of these movements. The first feature is concerned with thermonasty; the second, with photonasty. The opening of flowers of the tulip or saffron, *Crocus sativus*, which may be easily seen when these plants are carried from a cold into a warm room, is a typical instance of thermonasty. Under these conditions, the flowers will open in 3 to 5 min. (Fig. 135). The mechanism of this movement lies in the fact that with an increase of temperature the growth of the inner surface, morphologically the upper surface, overtakes the growth of the lower

surface of the petals, which then curve externally, and the flower opens. When the plant is left for a considerable period in a warm temperature, however, then the growth of the lower side also will be accelerated, and the flower will again close.

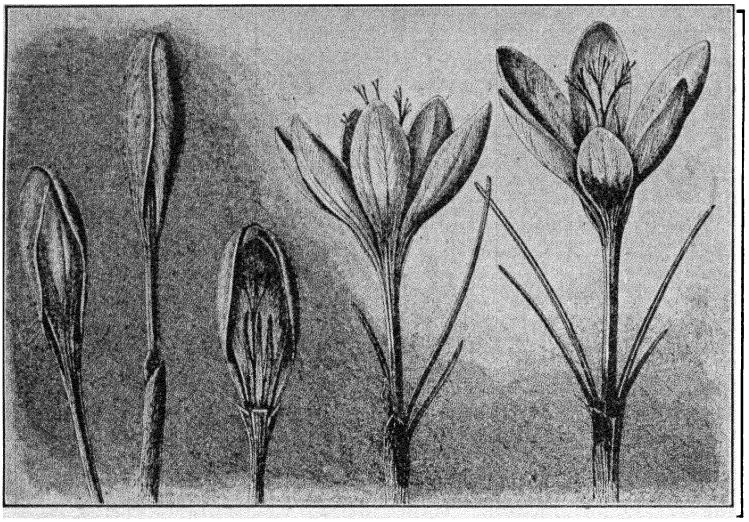


FIG. 135.—Thermonastic opening of saffron flowers (*after Kerner*).

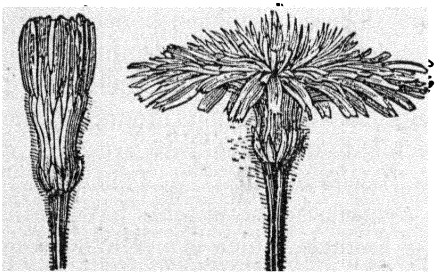


FIG. 136.—Photonastic opening of a dandelion flower head (*from Bonner Lehrbuch*).

The movements of petals of the water lily and of flowers of the dandelion are good examples of photonasty. Changes in the intensity of light make these plants close, not only in the evenings, but in dull weather as well (Fig. 136). This effect may be produced also by artificial shading, as for instance if the plant is carried into a weakly illuminated room. Some plants, like *Nicotiana* and the four-o'clock, on the contrary, close their flowers when the light is increased and open under weaker

illumination. They are fully expanded therefore in the evening and in cloudy weather.

Often the effect may be due to both temperature and illumination. The movements of the compound leaves of wood sorrel (*Oxalis*), clover, and other plants are thus conditioned. In some of these plants, the leaflets droop during the night; in others, they rise. These movements are produced by means of a special erecting tissue at the bases called the "pulvinus." The movements are of an alternating nature and are due to turgor changes in the upper and the lower halves of the pulvinus. Drooping movements are usually accompanied by an increase of turgor in the upper half of the pulvinus and a decrease in the lower half, while a lifting movement is produced by a reverse mechanism.

Nyctinastic movements of plants have an ecological role. In flowers, they facilitate pollination in favorable weather and protect the inner organs of flowers under unfavorable conditions. Nyctinastic changes in position of leaves are thought to be of importance as a protection against chilling at night. But since they are most common in the tropics where cooling at night presents no danger to the plant, this explanation should be accepted with reservations.

Of all the nastic movements, the greatest attention has been paid to those of seismonastic nature, which occur in response to a shock or concussion experienced by a plant.

Such movements may be observed in flowers of many *Compositae*, for instance, the cornflower. When touched, the threads of the stamens contract abruptly; this causes a lowering of the tube formed of united stamens surrounding the pistil and the throwing out of pollen onto the head of any insect that introduces its proboscis into the flower. In barberry, the staminal filaments curve rapidly when a special cushion of tissue at their bases is touched, and the stamens strike the pistil. All these movements are connected with the process of pollination of plants.

For other seismonastic movements not connected with flowering, the best-known illustration is supplied by the sensitive plant, *Mimosa pudica*, which rapidly drops its leaves when touched, all of its leaflets folding together in pairs (Fig. 137). If the plant is severely shaken, then all its leaves will drop at once. A very strong irritation of a separate leaf, as by injury

through cauterization or pinching, will result in a rather rapid transmission of the stimulus throughout the plant. As the reaction wave spreads through the branch, the leaves will close one after the other. This wave may be transported from one branch to another and thus finally will involve the whole organism.

If the temperature is sufficiently high, above 25 to 30°C., then the leaves of *Mimosa* will fold within a few seconds. Under these



FIG. 137.—*Mimosa* branch. On the left, two open leaves. On the right, a leaf lowered and folded, due to irritation (*after Nathansohn*).

conditions, the stimulus received is also transmitted with great rapidity, as fast as 15 mm. per second. Such an extremely rapid response is not characteristic of plants in general. Naturally then, *Mimosa*, which was first brought to Europe from Brazil in the seventeenth century, very soon became widely known not only in scientific circles, but among the public as well. The sensitivity of this plant is very much akin to the irritability of animals. It may be suppressed either by lowering the temperature to below 15°C., or by various anesthetics, especially the vapors of ether or chloroform.

What seismonastic sensitivity really consists in has not yet been discovered. The only thing so far established is that it is

unequally distributed through the plant and that the most sensitive part is the lower surface of the pulvinus, the swollen base of the petiole. The mechanism of the movement consists in a change of turgor in the upper and lower halves of the pulvinus, which supports the leaves (Fig. 137).

The stimulus suddenly increases the permeability of the protoplasm in the lower half of the pulvinus. The cell wall, which was formerly under considerable tension, becomes flaccid since the protoplast no longer presses against it when its permeability to water causes a decrease in volume of the protoplast. At the same time, part of the cell sap passes out into the intercellular spaces. This results in a rapid decrease of turgor in the lower half of the pulvinus. As the upper half preserves all of its tension, the petiole will bend downward, and the leaf droops. This extrusion of sap from the cells into the intercellular spaces is indicated by the lower half of the pulvinus becoming transparent. If a shock is produced in a leaf that has been cut off close to the stem and left to recover in a humid atmosphere, then it may be observed that the pulvinus curves and that a drop of liquid appears at its cut end. As the reaction passes, the liquid is reabsorbed by the leaf, and the pulvinus again straightens out.

100. Physiological Processes Accompanying Pollination and Fertilization. Germination of Pollen and Growth of the Pollen Tube. Phenomena Accompanying Termination of Blossoming. Shedding of Immature Fruit and Its Causes.—The process of fertilization and the subsequent development have been studied in great detail, both morphologically and cytologically. The physiology of the process, on the other hand, has been very little investigated. Because of its complexity, it seems to be difficult to subject the process to a detailed physiological analysis. Only a brief mention will be made here of the most important achievements in this field. The consideration of fertilization will be limited to the angiosperms, and the morphological and cytological side of the process will not be touched upon, since they are sufficiently described in textbooks of plant morphology and cytology.

Fertilization as such is preceded by pollination, *i.e.*, the transfer of pollen from the anther to the stigma, where the pollen germinates. In the germination of pollen, the external coat of the pollen grain (extine) is ruptured in definite places, while its

inner coat (intine) is stretched into a long tube that descends through the style, thus reaching the ovule. The pollen of the majority of plants can germinate not only on the stigma but also in artificial media, especially in sugar solutions at a concentration of 2 to 30 per cent. The pollen of a few plants can germinate in water. In the majority of plants, it will rupture and die when placed in water alone. Probably this is owing to the very high osmotic pressure that develops inside the pollen grain.

The substances necessary for the development of the pollen tube, nitrogenous as well as nonnitrogenous, are previously deposited in the pollen grain, and the length of the pollen tube is evidently determined primarily by the amount of these reserves. A possible supply of nutritive substances to the pollen tube from the tissue of the style is not excluded. Besides, the growth of the pollen tube requires the presence of some specific substance of the type of hormones or vitamins, which are supplied by the style. The pollen tubes of many plants will grow in the tissues of the style much better than in an artificial medium, in which they will make but feeble growth. In many instances, the pollen will germinate only on the stigmas of the same species; and when it does germinate on those of another species, the growth of the pollen tube will be so slow that it never reaches the ovule. The pollen of many plants that require cross-fertilization, as for instance fruit trees, produces very poor growth on the stigmas of the same individual, while it may grow excellently on other varieties of the same species. It is supposed that certain inhibiting substances are formed by the plant that delay the growth of its own pollen, thereby causing a physiological self-sterility in certain plants. It should be emphasized that this specific effect holds good for the pollen of all plants of the same clone, *i.e.*, individuals obtained by means of vegetative propagation from a single original individual. This fact is of great importance in horticulture and sometimes leads to low yields of fruit in an entire orchard.

The temperature conditions under which pollen germinates are also of considerable significance for successful pollination. There is a definite optimum temperature for the growth of pollen tubes, and the minimum is often quite high. Cold weather during blossoming time, therefore, has frequently an unfavorable

effect on fruit setting. The harm done by heavy rains and fogs is due to the fact that pollen subjected to protracted wetting with water will burst before germination. Besides, sugar and other substances necessary for the germination of pollen may be washed off the stigma.

Since pollen is a male gametophyte in a resting condition, it will preserve for a certain period its ability to germinate. The period of viability depends both on the specific properties of the plant and on the conditions of storage. The ability to germinate is preserved considerably longer in a dry, cool chamber. Under favorable conditions, for instance, when placed in desiccators, fertile pollen of some plants can be preserved for several months. The pollen of cereals and of some other plants represents a different physiological type. The membranes of these pollen grains are readily permeable to water. In dry air, such pollen rapidly desiccates and dies. It must, therefore, be kept in a moist atmosphere. But even under these conditions, its life is of short duration. The question of what factors help to prolong the life of pollen is also of great importance in the practical work of cross-fertilizing varieties that bloom at different times of the year.

Having reached the ovule or, more exactly, the embryo sac, the pollen tube ruptures, and the two generative nuclei contained in it effect fertilization. One of them unites with the egg nucleus, the other with the nuclei of the primary endosperm. When this has occurred, the division of the fertilized nuclei begins. The embryo develops from the fertilized egg, and the endosperm from the triple fusion nucleus. If fertilization has not taken place, this division usually does not occur, except in cases of parthenogenesis, which will not be discussed here. Hence the assumption has been made that some inhibiting factor prevents further development in a mature egg cell, which is removed by fertilization.

What this inhibition factor is and how it is removed by fertilization are problems that have not been sufficiently studied. Experiments with animals and with free-swimming egg cells of certain algae have shown that artificial parthenogenesis, *i.e.*, the division of nonfertilized egg cells, may be produced by means of various chemical stimulants, especially certain mineral salts. This leads one to the assumption that in higher plants the

generative nucleus of the pollen exerts a chemical stimulus that leads to further development of the egg nucleus.

Fertilization not only results in the development of the embryo and the endosperm but also in a number of other changes particularly in the ovule and other parts of the flower. The integuments of the ovule commence to grow and finally form the seed coat. The walls of the ovary develop and form the pericarp, while parts of the receptacle also are frequently involved in its formation. At the same time, the other organs of the flower, stamens, corolla, and often the calyx also, die and either drop off or dry up.

Since most of the parts of the flower are not directly involved in the process of fertilization, these changes must be regarded as a peculiar effect on the part of the developing embryo, or embryos, in case of polycarpic fruits. There is every reason to suppose that it is a matter of chemical stimulation. The fertilized egg probably secretes substances, which diffuse into the surrounding tissues and produce changes in their growth or senescence.

It should be noted that in certain plants withering of some of the floral organs occurs even before fertilization has taken place or at the time the pollen tubes begin to penetrate into the tissue of the stigma. This phenomenon is evident particularly in the orchids, where the nonpollinated flowers remain fresh for a long time but wither rapidly as soon as the pollen is transferred to the stigma. Fitting's experiments (1909) have shown that the same effect is produced when pollen that is incapable of producing fertilization is used. This seems to indicate quite clearly that special substances diffuse from the pollen and cause the withering of certain parts of the flower.

In some cases, there may arise chemical stimuli that will cause the growth of the ovary even when fertilization has not taken place and the ovule does not develop. Under such conditions, the plant will bear seedless fruits, which are characteristic of some cultivated plants such as the bananas, certain varieties of grapes, pears, and apples. This phenomenon is known as "parthenocarpy."

Usually, not all of the fruit set will ripen. Frequently, a considerable number soon cease to develop and are shed. Such a shedding of the fruit is especially frequent in fruit trees, in

cotton, etc. Very often, the fruit when shed has already reached a considerable size and may be close to complete ripening. The main cause of shedding of the fruit is a deficiency of nutritive substances, consumed rapidly when fruiting is simultaneous and profuse. Many crop plants produce more flowers and set more fruit than they are capable of maturing on account of the reserves stored and the nutritive substances that the leaves continue to elaborate; thus, very soon, an exhaustion of the reserves sets in. It is very probable that there is a deficiency not only in carbohydrates, proteins, fats, and such substances, but also in specific hormones controlling the movement of these substances toward the developing fruit. A struggle for the nutritive substances takes place between the individual fruits. The first that suffer are those that bore fewer or weaker seeds or are less favorably situated in respect to food supply. After they have ceased to obtain nutrients they drop off. Shedding represents an active physiological process. In the flower peduncle, there forms a special cork abscission layer, similar to that formed when leaves are shed in autumn. This layer grows across the tissues of the peduncle; when the abscission layer is complete, its cells break apart, and the fruit falls of its own weight.

As shedding usually occurs rather late, when a considerable part of the nutritive substances is already transferred to the fruit, it is accompanied by a useless expenditure of valuable substances. In intensive horticultural practice, attempts are made to avoid it by means of timely removal of flowers or very young fruit. If it is desirable to obtain especially large fruit, only a few of them are left to develop, all the others being removed. This can be accomplished best before the fruit has set. This method is widely applied in horticulture and gardening, in the cordon culture of fruit trees, debudding, and stake culture of tomatoes, etc. As the rapidly growing vegetative shoots are also consumers of nutrients, it is advisable not to allow an excessively vigorous growth of such shoots on fruiting plants. The removal of suckers from corn and of water sprouts from fruit trees is based on these considerations.

In an arid climate, shedding of the fruit may be the result of deficiency of water supply after the exhaustion of the soil moisture or after excessive expenditure of water during desiccating winds. Such a shedding is frequently observed, when cotton is

cultivated under irrigation, after an abundant irrigation in the beginning of summer, which promotes profuse flowering; if the plants remain for a long time without irrigation; or if a very severe drought occurs. In general, any abrupt change in the conditions of growth, especially if this change is disadvantageous and occurs during setting of the fruit, may lead to their abscission. Careful attention must be given to the plant at this sensitive period of life. Some fruits, such as peaches, plums, apples, and pears, have a periodicity in the shedding of their immature fruits that seems to be of a hereditary nature (Detjen, Murneck).

101. Ripening of Fruit and Seeds and the Biochemical Processes Accompanying It. Artificial Acceleration of Ripening.—Ripening of fruit and seeds is not merely the attainment of the proper size characteristic of a given variety of plants. A series of complicated morphological and biochemical changes occur in the fruit during ripening. The final stage of development of seeds is the attainment of dormancy, in which they may remain for a prolonged period until they are placed in conditions favorable for germination. In most plants, ripening of the seeds is accompanied by their gradual desiccation until they reach an air-dry state. The other parts of the fruit play only an auxiliary role in the process of reproduction. In dry fruits, they serve for the protection of the seeds. In succulent fruit, they attract animals, which consume them and thus contribute to the dispersal of the seeds, which in many cases are provided with a hard seed coat that allows them to pass through the alimentary tract of animals uninjured.

From a morphological viewpoint, ripening of the seeds represent the embryo, beginning from the fertilized egg cell and continuing till it becomes a small plantlet with its basic embryonic organs, the rootlet, the cotyledons, and the growing point of the stem. At the same time, food reserves are accumulated, which are necessary for the first stages of development of the young seedling before it has begun independently to absorb and assimilate nutritive substance from the surrounding medium. Their accumulation in the seeds will not be examined. The character of these reserves and their metabolism have already been discussed.

Fundamentally, the accretion of reserves in the ripening seeds represents somewhat the reverse of the processes occurring during

germination. Readily soluble and mobile substances, chiefly hexoses and amino acids, are transported toward the seeds and are transformed there into insoluble or almost insoluble substances of a high molecular weight, such as starch, fats, and proteins. The dry weight of ripening seeds increases rapidly. The water content, on the contrary, decreases continually. Toward the end of ripening, the loss in water may exceed the accumulation of dry substance and the weight of the seeds may even decrease. Novacky (1870) observed the following changes in the weight of ripening seeds of wheat in grams per 100 seeds.

Stage of development	Total weight	Dry weight	Water
Beginning of the milk stage	5.89	2.86	3.03
End of the milk stage	7.23	3.58	3.65
Dough stage	5.65	4.19	1.46
Completely ripe	4.59	4.22	0.37

The general changes in accumulation of the various substances in the wheat grain are presented in the following diagram (Fig. 138) taken from the work of Thatcher (1913).

In starchy seeds, the synthesis of this storage carbohydrate from monosaccharides proceeds very rapidly; and therefore, even in the early stages of development, the sugar content of ripening seeds does not exceed 1 or 2 per cent. In oily seeds, the metabolism of carbohydrates into fats is more complicated. Very often in the early stages of ripening there are found in these seeds not only monosaccharides, but even starch, which are gradually transformed up to the time of complete ripeness into fats. Leclerc du Sablon observed the following changes in the content of different substances of the walnut. On July 6, the seeds contained 3 per cent of oil, 7.6 per cent of sugars, and 21.8 per cent of starch and dextrin. On August 1, the oil content rose to 16 per cent, while the sugar content fell to 2.9 per cent and that of starch of 14.5 per cent. At the time of complete ripeness, October 4, the oil content increased to 62 per cent, while the sugar content fell to 1.5 per cent and that of starch to 2.6 per cent.

As has been shown by S. Ivanov, the character of the oil, also, changes during ripening. In the beginning of this process, saturated fatty acids prevail; later, the unsaturated acids increase in proportion until they become more abundant, and toward the end of ripening, the seeds are filled with neutral fat

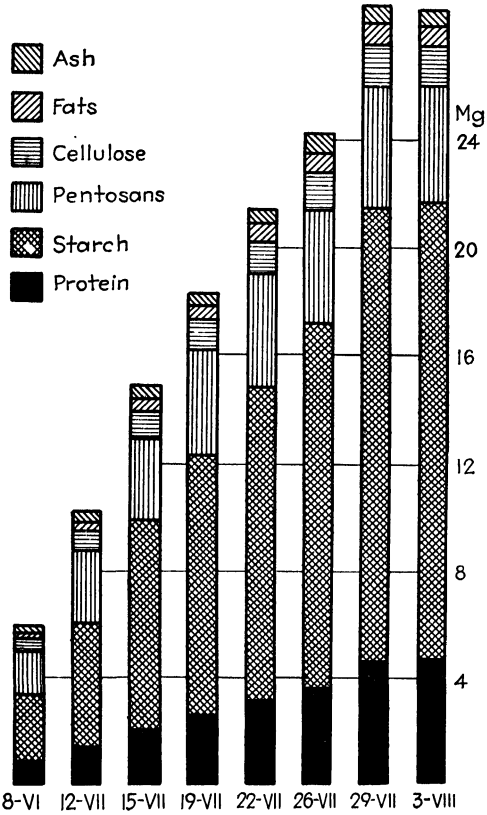


FIG. 138.—Course of the accumulation of substances during the ripening of wheat grain.

of mostly unsaturated acids. He presents the following scheme, based on these data, for the formation of fats in ripening seeds:

carbohydrates → glycerin ——— ↘ oil
carbohydrates → saturated fatty acids → unsaturated fatty acids ↗ oil

From Thatcher's data, it follows that ash is accumulated during ripening. As it proceeds, the quantitative relations

between the separate elements change substantially. The potassium content decreases, while that of magnesium and especially of phosphorus increases. This large accumulation of phosphorus in ripening seeds explains the great influence of phosphate fertilizers upon the yield of grain. Nitrogenous fertilizers, as has been noted previously, increase the yield of the total dry weight of the plant. Phosphorus penetrates into the seeds in the form of inorganic phosphates but is deposited in the form of organic phosphorus compounds, chiefly lipoids and phosphatides.

The ripening of fleshy fruits is accompanied like the ripening of seeds, by an accumulation of nutritive substances, but the latter serve not for the nutrition of the embryo, but for the attraction of animals, which disseminate the seeds. Substances with a palatable taste predominate in fruit, chiefly sugars, pectin compounds, starch, organic acids, and flavoring esters. Usually, the ripening of the seeds coincides with the ripening of the pericarp. So long as the seeds are not mature, the pericarp is acid or astringent in flavor owing to the presence of a large amount of organic acids and tannins. With ripening, these substances are replaced by sugars. At the same time, essential changes occur in the physical properties of the fruit. Previously hard, they now become soft, this being a result of the change in nature of the pectins, which cement together the cells of the flesh. Under the influence of enzymes, they become soluble in the water impregnating the cell walls; the cells lose their firm intercellular cement, and their shape changes from polyhedrons to become more or less spherical. Such a softened tissue can be more readily eaten by animals and more easily digested by the alimentary juices. Other changes in structure and chemical composition of the cell walls and cell contents increase the palatability of fruits.

In the first stages of development of apples and other succulent fruits, the sugars translocated from the leaves are deposited in the cells of the flesh of the fruits in the form of starch. This contributes to a slight extent to their hardness and to the absence of a sweet taste. In later stages of ripening, this starch is transformed into sugar, the amount of which increases rapidly in the fruit. In the so-called "winter varieties," these transformations take place only after the removal of the fruit from the tree,

during the time of storage when there can be no more influx of carbohydrates.

When storage is prolonged, processes of overripening and death occur in the cells of the flesh, the activity of proteolytic enzymes increases, and autolysis begins. The connection between the cells is lost, and the flesh is transformed into a soft pulp. Such overripened fruits are readily attacked by molds and bacteria.

The transformation of starch into sugar and the oxidation of tannins and organic acids, which proceed slowly in the case of natural ripening, may be accelerated artificially by means of special stimulation. The method of treating mature unripe fruit, chiefly bananas and tomatoes, with ethylene gas, elaborated in America by Harvey, has obtained wide practical application. The treatment is carried out during 2 to 3 days in tightly closed chambers containing an insignificant amount of ethylene, about 1 part of ethylene per 1,000 parts of air. The effect of ethylene is explained by the fact that it hastens enzyme action and may increase the permeability of the protoplasm and thus facilitate the access of oxygen. This increases the oxidation processes leading to a more rapid transformation of tannins and organic acids. An increase of permeability also promotes the diffusion and activity of the enzymes, this likewise contributing to the digestion of starch and other higher carbohydrates and of the pectinous substances that hold the cells together and thus leading to a softening of the fruit. Besides the addition of ethylene, it is necessary to keep the temperature at about 18 to 21°C. and the relative humidity of the air not below 90 to 95 per cent. The same effect is produced on fruit by propylene and to a considerable degree by acetylene.

Artificial ripening of the fruit differs from the natural process in that the seeds do not accelerate their development. The changes occur principally in the cells of the flesh of the fruit.

In ripe fruit, the seeds that are also ripe and consequently ready to germinate are frequently surrounded by a watery medium, and the question arises as to why germination is not observed inside the fruit. The absence of germination may be determined by various causes. In many succulent fruits, *e.g.*, in apples, pears, and especially in hawthorn berries, the seeds of even completely ripe fruit have not quite finished all the processes preparatory to germination and require a rather prolonged period

of time before they attain the ability to germinate. As is known, they must be subjected to stratification, which consists in their being kept for several weeks in a moist state at a temperature slightly above 0°C. In other plants, as for instance in watermelons, the flesh of the fruit contains special substances of the type of hormones that inhibit germination, which becomes possible only after the release of the seeds from the flesh. This occurs under natural conditions when animals devour the fruits. Germination is also inhibited by the comparatively high cell-sap concentration of the flesh.

When fruits are overripe, these inhibitive factors are removed, and the seeds become capable of germinating. But sometimes such inhibitive factors for some unknown cause are absent in succulent fruits, and then mass germination of seeds occurs inside the fruit that has not yet completely ripened, as is the case with the citrus and many tropical fruits.

102. Influence of Environmental Conditions in the Course of Ripening. Shriveled Grain and Its Causes. Dependence of the Chemical Composition of Fruit and Seeds on Geographical Factors.—The successful filling and ripening of grain depend to a great degree upon the environmental conditions during this period. Cases are known when prospects of a good yield were entirely shattered in consequence of unfavorable weather conditions.

High temperatures and dryness of the air with high winds bring great disasters to agriculture in the southeastern and eastern provinces of Russia and in the Great Plains of the United States, owing to poor filling of grain. Two or three days or sometimes even a few hours of such dry winds may induce premature ripening of the grain, which remains insufficiently filled and shrivels, the yield thereby being decreased to one-half or one-third. The main cause of this phenomenon is the disturbance of the normal flow of nutrient substances from the leaves to the ripening seeds. A sufficient turgidity of all the organs of the plant, especially of the leaves, which are the source of carbohydrates, is necessary for producing this normal flow. According to Münch's scheme, examined in Art. 79, the prime mover of the current of elaborated substances is the turgor pressure of the cells that form the assimilates; and when this tension falls during dry winds, the translocation of assimilates

into the ripening grain ceases. At the same time, the delivery of water is likewise inhibited, the grain dries, and ripening occurs prematurely. It is important to note that after the dry wind has ceased, the wind-burned plants are no longer capable of restoring the normal supply to the seeds because of some irreversible changes that are not understood as yet and have not been investigated.

Shriveling may be the result not only of a disturbance in water supply of the plants; rust and other parasitic fungi, utilizing the carbohydrates elaborated by the leaves and thus inhibiting their translocation into the grain, also cause a shrinkage of the grain. The same results are produced by the parasitic higher plant *Orobanche*, which develops on the roots of sunflowers. A sharp diminution of the assimilating leaf surface from defoliation caused by caterpillars, locusts, and other insects leads to analogous consequences.

Premature ripening because of wind burn is also accompanied by changes in the chemical composition of the grain. As it desiccates in its early stages of development, the processes of transformation of soluble carbohydrates into starch are not completed, and consequently instead of a starchy grain whose cells are filled with starch, there results grain with a glassy fracture, in which the starch grains are held together in a horny mass by dextrines. The accumulation of proteins is reduced less than that of carbohydrates. Hence, wind-burned grain is relatively richer in proteins than well-filled grain. But though it may contain a higher percentage of proteins yet per 1,000 grains, it is considerably poorer in nitrogen.

In arid regions, especially on slightly saline soils, when the conditions of water supply are not favorable on account of the high osmotic pressure of the soil solution, the ripening of grain even in the most favorable years always proceeds under conditions that impede its filling, and the grain is somewhat poorer in starch but relatively richer in proteins than in more humid regions. Such a change in the chemical composition of the grain of arid regions makes it especially valuable for baking bread and especially for macaroni production. The wheat from the semi-arid regions of the lower Volga in Russia and from the Dakotas has been highly esteemed in Italy and other European countries and has formed the chief supply for macaroni.

The high protein content of wheat resulting from an impeded water supply is usually decreased with the application of artificial irrigation, which contributes to a better filling of the grain with starch. But as with irrigation the plants nevertheless continue to endure atmospheric drought, the grain of irrigated wheats remains richer in proteins than that of wheat grown in humid climates and thus retains its higher baking qualities. Besides, it is possible to produce a higher protein content by means of introducing nitrogen fertilizers with the irrigation water, especially in the second half of the vegetative period or toward the time of the filling of the grain. It is also possible to produce a higher protein content by means of selection; for it has been observed that, in some varieties, irrigation decreases the protein content of grain less than in others.

Climatic conditions produce an analogous effect upon the protein content of barley. Here, likewise, a more arid continental climate contributes to an increase of the protein content, while a more humid maritime climate contributes to its decrease. Therefore, barleys from less arid regions are more suitable for brewing, in which a low protein content is desired; while barleys from arid regions are better for feeding purposes. Likewise, the climate of high altitudes, differing from the climate of valleys in increased humidity and lower temperatures, produces barley more suitable for brewing.

The comparison of the chemical composition of grain from different regions and districts cannot give a sufficiently clear picture of the dependence of its composition upon geographical factors; for different varieties are sown in different regions, and this alone may account for the variations in the chemical composition, even when the plants are grown in a similar environment. To get an idea of the significance of geographic factors, the same varieties must be grown in different regions. This has been done in extensive geographical experiments carried out by the Institute of Plant Industry in Russia under the direction of N. I. Vavilov. Those experiments have shown that the same varieties sown in different geographical positions change very abruptly the composition of their grain.

Climatic conditions affect greatly the chemical composition likewise of oily seeds. The total fat content does not change significantly in different geographical regions, but the chemical

composition of the fat changes considerably. According to the investigations of S. L. Ivanov, a certain variety of flax gave in the Moscow region excellent drying oil due to a high content of the more unsaturated fatty acids, containing three double bonds such as linolenic acid, while in Turkestan the same variety produced oil with a prevalence of less unsaturated acids, yielding a poor drying oil. If during ripening of the seeds there prevail lower temperatures with alternation between the day and night temperatures, then mostly unsaturated acids are formed. But on the contrary, if ripening occurs at higher and more uniform temperatures, more saturated acids are produced. Mountain regions are similar to northern regions in their effect upon oil plants. Hence, the practical conclusion may be drawn that the best varieties of drying oil are obtained from oil seeds either of northern or of high mountain origin.

Climatic conditions also exert a great influence upon the ripening of succulent fruits, temperature being likewise the chief factor. With low temperatures and a prevalence of cloudy weather, which reduces the heating of the fruit by direct sunrays, they ripen slower and contain more acids and tannins and less sugars. Conversely, in a warm and sunny climate, the fruit produced is richer in sugars. But, of course, varietal differences in the chemical composition are of great significance in this respect.

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CHAPTER XIV

SEASONAL PHENOMENA IN THE LIFE OF PLANTS

103. The Dormant Period of Plants. Autumnal Maturation of the Buds and Wood of Trees. Shedding of Leaves.—In the evolution of plants, the periodic alternation of the seasons of the year with a periodic return of winter, which stops the growth of plants and kills their tender organs, has left a deep impression on the life habits of perennial plants, especially of woody species. Periods of intensive growth in summer alternate with an almost complete suppression of all vital processes in winter. It is only plants native to the perpetually humid tropics that are capable of vegetating uninterruptedly throughout the year. It is precisely this peculiarity that has made them suitable as greenhouse crops. Plants of a temperate climate stop growth during winter, shed their leaves and sometimes all aerial shoots, and then enter into a state of rest, or dormancy.

One must not imagine that in this state there is a complete cessation of all vital processes. Resting organs, *e.g.*, the buds of trees and the tubers and rhizomes of grassy perennials, respire during complete dormancy. Transformations of reserve substances also occur. For instance, in the twigs and buds of trees, starch gradually disappears and is replaced by sugars and fats, leading to an increase of frost resistance. Toward the spring, the reverse metabolic changes may take place. In general, all vital activities manifest themselves during dormancy with the exception of growth, which does not take place, though the external conditions may be quite favorable to it. One cannot make the potato tuber sprout in September and October even under proper temperature and moisture conditions, while in spring it will develop in a cold dry room.

The period of dormancy very often may be separated into two stages: the stage of meristematic growth and that of elongation. Such a rest period may occur even in annuals, especially in

cereals (Fig. 139). A detailed study of the growth of wheat, for example, shows that during the first weeks after germination of the seeds, the stems remain short, forming the so-called "tillering node," when the leaves alone develop. But when the internodes and the inflorescence have completed their embryonic development, then a rapid elongation of the internodes, or shooting, begins. The inflorescence is pushed out of the tubelike leaf sheaths, which until then have tightly enclosed it.

In biennials, these two growth stages are separated by the winter period, when the plant has entered into a state of rest.

The transition of trees into a state of rest, which generally occurs in autumn, is usually accompanied by a shedding of

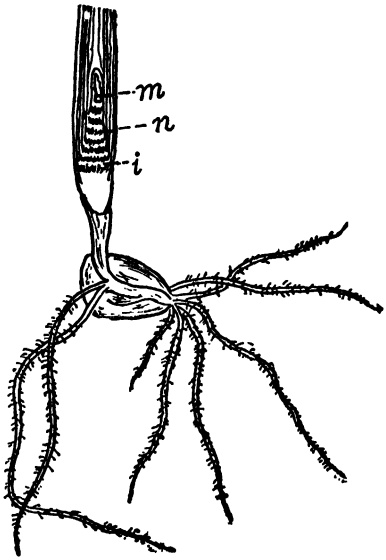


FIG. 139.—Terminal bud of a young maize plant.

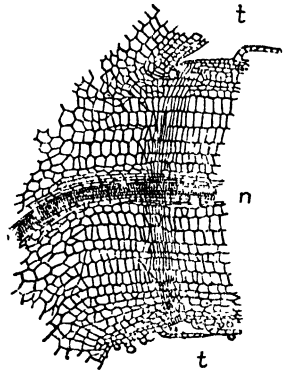


FIG. 140.—Abscission layer formed across a petiole at *t* on each side of vascular bundle *n*.

leaves and sometimes of whole twigs, mostly the shorter ones. This shedding represents a normal physiological process and is caused by the formation of a special secondary meristem, the so-called "abscission layer" (Fig. 140) in the leaf peduncle. The cells of this layer become rounded and separate from each other, the leaf remaining attached only by means of the bundle of conductive elements. Finally, they also rupture, and the leaf is shed.

The causes of the formation of an abscission layer in the period of leaf fall are not clear as yet. At any rate, it is not the direct

influence of frost, for our trees shed leaves in autumn even when kept in a room or warm greenhouse. The investigations of Garner and Allard (1920) have shown that a great influence is exerted by the length of the day, which rapidly decreases toward the autumn. By artificially shortening the summer day and prolonging the night by covering the plants with dark boxes or moving them into a dark chamber in the morning and evening hours, Garner and Allard observed in many tree species a very early leaf fall. These observations were considerably expanded by Moshkov (1930), who has been able to show that not only abscission of leaves but all the processes of the so-called "autumnal maturation" of the shoots, such as the cessation of growth, the dormancy of the top bud, the development of a cork layer on the surface of the shoots, and the accumulation of reserve substances in the bark are controlled to a great degree by the relative length of the day and night. If this relationship is changed, the time when trees begin their winter dormancy can be controlled within wide limits.

Dormant organs and tissues of plants as compared with those in a state of active growth are much more resistant to unfavorable external conditions, especially to frost and drought, owing to a decreased water content, an increased amount of reserve substances, and changes occurring in the colloidal state of the protoplasm. Both abscission of leaves and ripening of the wood are reactions of the plant in response to the beginning of drought in tropical climates and to the beginning of winter in moderate latitudes. Likewise, cereals during the period preceding the elongation of the stem, when the organs of reproduction are still in an embryonic resting state, are more drought resistant than later on when the stem and spike begin to elongate. Thus, the rest period may be regarded as a special adaptation fixed by inheritance for surviving unfavorable seasons of the year. By controlling the beginning of this period, Moshkov was able to control the frost resistance of tree species. Such southern species as *Robinia pseudoacacia*, walnut, and apricot, which usually freeze in Leningrad, overwintered excellently without any protection when grown with a shortened day. This explains the fact why *Robinia pseudoacacia* overwinters well in the southern Volga region, where the winter is more severe but the days are considerably shorter than in Leningrad.

104. Artificial Breaking of the Rest Period. Forcing of Plants. Significance of This Method for Agricultural and Floricultural Practice.—The causes that inhibit the growth of dormant plant organs, are not known. Many authors suppose the presence of hypothetical growth-inhibiting substances. For instance, the dormancy of lateral and accessory buds and their rejuvenation after the removal of the terminal bud are explained by the assumption that the growing point sends downward hormones that inhibit cell division. After removal of the top bud, the inhibiting influence ceases.



FIG. 141.—Lily of the valley treated with ether, *A*, and control, *B* (*after Duggar*).

In this case, there is a manifestation of correlation between the separate parts of the plant, which was discussed in Art. 91.

But even if the internal mechanism of dormancy is not yet understood, nevertheless there are many means of breaking it artificially. Most of these methods have originated in horticultural practice, and their perfecting has been promoted by the desire to obtain flowering plants in winter, which are highly valued but difficult to force at this time. Recently, new and improved methods of breaking the rest period have been established by physiologists. The most important of these is treatment with ether, discovered by Johannsen; the exposure to warm baths, suggested by Molisch; and the newer methods practiced in the United States, using more efficient and less toxic chemicals.

Plants intended for treatment with ether, such as flowering shoots of lilac and rhizomes of lily of the valley, are placed in airtight wooden boxes with closely fitting doors; in these is also placed a small amount of ethyl ether. The concentration of ether used is 0.5 cc. per liter of air. The plants are allowed to stand in this vapor for 24 or 48 hr., after which they are transferred to a warm greenhouse. Soon the treated plants will open their buds and begin to bloom, while the controls, though receiving an identical treatment except for being subjected to the action of ether, will remain dormant (Fig. 141).

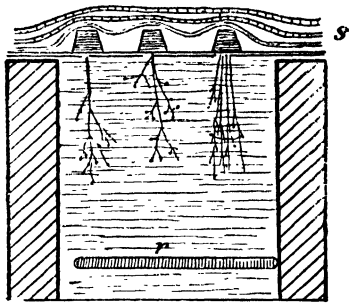


FIG. 142.—A tank for warm baths, heated from below by a radiator, *r*, and covered with felt, *s* (*after Molisch*).



FIG. 143.—Shoot of lilac. Right branch subjected to a warm bath, left one remained untreated (*after Duggar*).

Exposure to a warm bath is still simpler. The aerial parts of the plants are immersed for 9 to 12 hr. in water at 30 to 35°C.

(Fig. 142) and then are transferred to a place favorable for growth. It is interesting to note that the effects of warm baths and of ether are purely local. Accelerated development takes place only in buds and branches that have been exposed directly to the stimulants. When a lilac bush is placed in such a position that only part of its branches are in warm water, the others remaining outside, then in a few days a marked difference between the development of these branches will be observed (Fig. 143).

Besides the treatments with ether and warm baths, several other methods used to induce the buds of plants to break their rest period have been recently discovered. Plants may be forced by various volatile substances, such as hydrocyanic acid, acetone, ethylene, illuminating gas, tobacco, and other fumes. In Puerto Rico, it is a common practice to smudge fields of pineapples to force them into bloom. Cutting of the buds, injection of water or salt solutions, exposure to X-rays and radium emanation, and many other external influences will hasten the breaking of buds. Also, a considerable acceleration in development will be observed after freezing. Plants that have remained during the winter in a warm room will start to grow later than those subjected to the action of frost (Fig. 144).

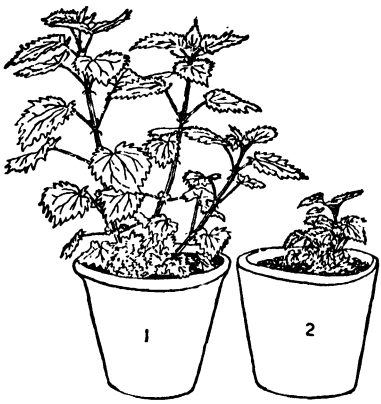


FIG. 144.—Nettle kept during the winter (1) under snow, (2) in a warm greenhouse.

Of the greatest practical importance are the methods of breaking the rest period of potato tubers. In many southern regions, the vegetative period is long enough to obtain two successive yields of potatoes during one summer, at least of the earlier varieties, but this is seriously impeded by the fact that young tubers remain dormant for several months after harvest. Most fall-harvested potatoes are dormant until January or February. By treating potato tubers with different chemical agents, thiourea, thiocyanates or ethylene chlorhydrin, Denny was able to produce rapid germination of freshly harvested potatoes. Of all

these substances, ethylene chlorhydrin is the most practical, owing to its cheapness and convenience in manipulation. The tubers are soaked for several minutes in dilute solutions. In Russia, experiments with ethylene chlorhydrin have been successfully carried out at several experiment stations. Treatment with ethylene chlorhydrin not only breaks the rest period of potato tubers and accelerates their germination, but it likewise contributes to earlier tuber formation in plants that have developed from treated tubers. Northern-grown seed potatoes are used in the coastal Gulf States for planting after their rest period has been broken. This may be done by sprinkling ethylene chlorhydrin in the railway cars after loading.

The mechanism of the effects of these stimulants is unknown. Some authors are of the opinion that they increase the permeability of protoplasm and thereby induce growth. Others hold that they increase the oxidation processes in the cell and thus stimulate its vital activities. By studying the effects of ether treatment at different times in the fall and winter, Johannsen came to the conclusion that its influence is not the same at different periods. This he considers an indication that the state of rest likewise varies. He distinguishes three conditions of rest: a preliminary one, a stage of deep rest, and a condition of enforced rest, which gradually and imperceptibly merge one into another. During the preliminary stage, through which most buds pass in summer, they can be readily returned to active growth. This may be accomplished simply by removing the leaves. In some trees, such as oaks and elms, the buds frequently open in summer, even without special treatment, and produce secondary growth. In the fall, after the leaves have normally dropped, bud dormancy is in its deepest stage. At this time, forcing requires particularly strong stimulants. Toward late winter and early spring, buds gradually leave the state of rest. When placed under conditions favorable for growth, they will begin to unfold without any particular treatment. The stage of enforced rest and the absence of growth at this period are no longer determined by internal conditions but solely by unfavorable environmental conditions. During this period, the usual stimulants will not hasten the unfolding of buds.

105. Conditions of Seed Germination.—The mature seed generally has a period of rest. Germination necessitates a change to active growth. As in buds, this resting condition

may be light or deep. Accordingly, many seeds germinate as soon as they are placed under favorable conditions of moisture and temperature, while others will not germinate under the same conditions for many weeks, months, or even years.

Various causes are responsible for this delay in germination, just as there are manifold methods by which germination may be hastened. Very often there is exhibited the same phenomenon of deep rest or dormancy that has just been discussed in the consideration of buds. In such cases, seeds acquire the capacity to germinate rapidly only after having been stored for a sufficiently long time, or else they require some external stimulation.

A gradually increasing power of germination is often found in winter wheat and other cereals, which sometimes appear incapable of germinating immediately after harvesting. A few weeks or months later, however, they can be germinated quickly and uniformly. This phenomenon has been termed "after-ripening." This characteristic is exhibited by various varieties in different degrees. In regions with a very wet autumn, varieties of wheat with a long rest period may be of advantage in that their grains will not germinate in the shock or stack in rainy weather. Larson, Harvey, and Larson have recently made a study of the length of the rest period of cereal varieties, which shows that the time of cutting, the temperature of storage, and varietal inheritance are important in determining the length of the rest period.

On account of the necessity of seeds to pass through a rest period, there have been developed methods of stratification, which are widely used in horticulture. The mature seeds of the apple, pear, and stone fruits are usually planted in layers with moist sand and transferred to a cold place, for instance to a cellar, the best temperature being +5°C., or they may be left under snow. Toward spring, the rest period will be completed, and the seeds will germinate. Instead of being buried in moist sand, the seeds of apples, for instance, may be kept simply by leaving them in the flesh of the fruit. In dry seeds of these plants, the process of afterripening does not take place.

The rest period of mature seeds may be so deep that they will refuse to germinate without exposure to special stimulation even after prolonged storage. The seeds of the gooseberry, *Grossularia* sp., of *Lunaria*, of *Impatiens*, and other plants require more than a simple stratification. They must be frozen at

−5 or even −10°C. to induce germination. Light is a stimulant to many seeds that fail to germinate or that germinate but poorly in darkness. To this group belong seeds of *Poa*, tobacco, carrot, *Oenothera*, and many other plants. Contrariwise, light retards germination of the seeds of some plants, like those of the thorn apple, *Crataegus* sp., of *Amaranthus*, etc. Hence, they must always be germinated in darkness. What the stimulating effect of light on the germination of seeds consists in is as yet quite obscure. The action of light may be replaced by fluctuating temperature, within the range of a few degrees, or by various chemicals, among which the most important are the acids and alkalies. Seeds of bog plants, like *Alisma*, *Sagittaria*, and others, will germinate only in an acid medium. Those of certain parasites, like the broomrape *Orobanche*, also require an acid medium. Others are stimulated by solutions of neutral salts or substances like bromine, iodine, and certain antiseptics.

The increasingly popular treatment of seeds with various solutions, such as Formalin, copper sulphate, and various organomercury preparations, in order to control smut and other fungus diseases, sometimes results in stimulated germination, but frequently it leads to retardation.

A prolonged delay of germination does not always depend on the state of dormancy in which the embryo of the seed happens to be. Very often it is due to the properties of the seed coat. Many plants produce so-called "hard seeds," which fail to germinate because their coats are impermeable to water. Such seeds very often occur in clover, alfalfa, lupine, and other representatives of the family *Leguminosae*. If a certain number of seeds of such plants are placed in water, part of them will rapidly swell and germinate, but others will remain for a long time in water without swelling. Only gradually, after some weeks or months, will a few seeds absorb water and germinate, apparently, as a result of a lesion in the outer layers of the seed coat. Molisch observed in one of his prolonged experiments with the seeds of the Japanese *Gleditsia* that 4 seeds out of 57 placed in water had swollen on the next day; 11 more, within the first 2 months; 21 additional, during the first year; 6, in the second year; 6 more, during the third year; 3 in the fourth, and 3 in the fifth year; while 3 seeds did not swell after having been in water for more than 5 years.

The impermeability of the coats of hard seeds to water depends on very close-packed dry thick-walled cells forming the outer layers of the seed coat. When these outer layers are injured by scratching with a knife, by filing, or simply by scratching them with sand, swelling and germination will result. The corroding action of strong sulphuric acid also may be used for this purpose.

Not always, however, is the delayed germination of viable seeds due to the impermeability of their coats to water. Very often a nonpermeability of the seed coat to gases is the real cause, because of which the carbon dioxide produced during respiration is not eliminated but accumulates within the seed and inhibits the active growth of the embryo. Such seeds may be kept for years without germination, though they swell readily. For seeds of many of the common weeds, like plantain, black mustard, shepherd's-purse, wild oats, and chickweed, it is not sufficient merely to scratch their seed coats. They must be split to permit the escape of carbon dioxide and admit oxygen. Seeds that happen to be covered by a thick layer of earth may be in a similar condition. They may remain alive in the ground for many years without germination but will germinate rapidly as soon as they have reached the top of the soil. For example, in samples of soil taken from a depth of about 20 cm. in a forest growing on ground that 30 to 40 years before had been occupied by a field or meadow, seedlings of weeds or meadow plants could be obtained. Evidently the seeds had retained their power of germination throughout this long period. Seeds of *Nelumbo* have been found viable after more than 100 years under certain conditions.

Such long preservation in moist ground without decaying is possible only because the seed coat excretes on its surface some antiseptic substances that inhibit the development of bacteria and molds. It is interesting to note that in dead seeds this resistance to rots soon disappears, and they rapidly become the prey of microorganisms that produce decay.

As the seed matures, the impermeability of its coat gradually increases, and desiccation often makes it still more impermeable. This explains the commonly observed phenomenon that in a fresh supply of seeds the percentage of hard seeds is somewhat lower than in those that have been stored for a long time. It

also accounts for the fact that unripe seeds of many weeds germinate better and more rapidly than ripe ones. The ready germination of immature seeds, which normally pass through a period of rest, as for instance the seeds of winter wheat, is probably due to the fact that they are in a condition of preliminary rest, which is more readily disturbed than a deep rest.

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INDEX

A

Aamodt, 372
Abderhalden, 53
Abscission of fruit, 363
Absorber of Ordojan, 164
Absorption, of mineral salts by plant roots, 253
  of mineral substances at different periods of development, 259–261
  of water by the plant, 21–25
Absorption spectrum of chlorophyll, 176
Acceptors, of hydrogen, 102
  of oxygen, 104
Acetaldehyde, 98–99
Activators of enzymes, 67
Active acidity of the soil, 245–247
Activity of enzymes, 66
Adsorbing capacity of soil, 253–255
Adsorbing soil complex, 253
Adsorption, 4, 253–255
Aeration of the soil, 252
Aerotropism, 157
Afterripening of seeds, 460
Albinism, 175
Albumins, 54
Alcoholic fermentation, 97–101
Aleurone grains, 50–51, 79
Alpine plants, 135
Aluminum, toxicity of, 272
Amino acids, 2, 29, 50–55, 73–78
Ammonium in plants, 73–74
Ammonium ion, toxicity of, 224
Ammonium nitrogen, 220
Ammonium oxidation in the soil, 181
Amylase, 56, 70
Amylophilous plants, 163
Anaerobic processes in bog soils, 376–377
Anaerobic respiration, 96–97
André, 267
Antagonism of ions, 7, 140–141
Anticnzymes, 67
Apparatus, Pettenkoffer's, 86
Arrhenius, 270
Artificial fertilizers, 235, 263
Ascent of water in the tree trunk, 320
Ash in plants, 239–240
Ash elements indispensable for plants, 240–245
Askenasy, 330
Asparagine, 73–77, 226
Assimilates, 162–164
Assimilation, of carbon, 161–167, 194
  of free nitrogen, 228–231
Assimilation number, 180
Atmospheric factors influencing transpiration, 301–302
Autolysis, 65, 69–70
Autotrophic plants, 223
Autotropism, 158
Auxin, 123–127
Auxographs, 119
Available and unavailable soil moisture, 289–290
Azzi, 373

B

Bach, 70, 72, 103–104
Bacteria, hydrogen, 181
  nitrification, 182
  nodule, 232–235
  sulphur, 183–184
Bacteria-fixing nitrogen, 229–231
Bacteria-oxidizing iron salts, 184
Baeyer, Von, 184
Balanced solutions, 139–142
Beijerinck, 230

Biuret reaction, 51
Blackman, 84, 94, 101, 122
Blackman's reaction, 187, 204
Bleeding of plants, 284
Blooming, 410
Bog soils, 377–378
Bog toxins, 376
Bogdanov, 290
Bonnier, 84, 135
Borodin, 84, 93
Bose, 119, 336
Bourquelot, 64
Boussingault, 73
Boysen-Jensen, 124, 197
Briggs, 290
Brown, 190
Browner, 149
Brownov, 373
Buchner, 98
Budding, 404
Buds, dormant, 396–397, 401
Buffer action of solutions, 272
Butkevitch, 262

C

Cacti, 366
Calciophiles and calciophobes, 268
Callus, 321, 401, 415
Cambial cell, 342
Candolle, De, 154
Cannizzaro, 102
Capillary water in the soil, 288
Carbon dioxide content of the air, 201
Carbon dioxide cycle in nature, 201–203
Carbohydrases, 68
Carbohydrates, reserve, 36–45
Carboxylases, 100
Cardinal points, of enzymes, 66
  of germination of seeds, 129
  of growth, 129
  of photosynthesis, 204
  of respiration, 92
Carotene, 168, 171–173
Catalase, 101, 188
Catalysts, 61
Cations of heavy metals, toxicity of, 141
Cellulose, 9, 45–46
Centrifugal force, 147–148
Chemical composition, of grain dependent on climate, 359–361
  of protoplasm, 34–37
Chemical processes during photosynthesis, 184–186
Chemistry, of alcoholic fermentation, 101–102
  of respiration, 84–86, 89–91
Chemosynthesis, 181
Chemotropism, 155–157
Chimeras, 403
Chlorophyll, 167
Chloroplasts, 168–169
Chlorosis, 173–175
Cholodny, 125–127, 149
Chromogens, respiratory, 104
Citric acid, 49
Classification, of enzymes, 66
  of protein substances, 54
Clinostat, 146
Coagulation, 6, 28, 352
Coefficient, wilting, 289
Coenzymes, 67
Cohesion theory, 332
Cold resistance of plants, 355–358
Combes, 195
Compensation point, 197–201
Compression of vessels during ascent of water, 332–334
Concentration of hydrogen ions, 257, 352
Conditions of chlorophyll formation, 168–169
Constitutional proteins, 29
Content of sugar in overwintering plants, 353
Critical period, 373
Crone's solution, 257
Cultures, in electric light, 198–201
  pot, method of, 258–262
  water, 251–254
Cuticle, 189, 304, 385
Cuticular transpiration, 303–305
Cuttings, 399

Cycle, of carbon dioxide, 201–203
of development, 409–412
of nitrogen, 235–237
of phosphorus, 246
of sulphur, 245–246
Cytase, 58

D

Daily march of stomatal movements, 308
Daily march of transpiration, 302
Daily periodicity of growth, 135
Dalton's law, 301
Darwin, 148, 158, 307
Desamidase, 73
Descending stream of assimilates, 321–322
Desmolases, 68
Destruction of chlorophyll in the light, 172
Detjen, 442
Dewar's flask, 88
Dialyzer, 5
Dialysis, 4
Diastase, 56, 70
Diffusion, 12
Diffusion of gases, 190–191
Disperse phase, 3, 10
Dispersion medium, 4, 10
Dissolving capacity of the root, 255–258
Diurnal march of photosynthesis, 208–210
Dixon, 332
Donnan's equilibrium, 30–31, 252
Dormancy, of plants, 453
of seeds, 459–463
Dormant buds, 396–397, 401
Drought, atmospheric, 363–364
soil, 364–365
winter, 350
Drought resistance, 370–372
and earliness, 372
Dry winds, 365–367
Duration of plant life, 350
Dutrochet, 13–14, 285

E

Efficiency of radiant energy, 179–180
Egiz, 426
Electrophoresis, 5
Endosmosis, 13
Engelgard, 257
Engelmann, 168
Enzymes, 56, 61
Ereptase, 61
Escombe, 190
Etherization, 456–457
Etiolation, 132–133
Eudiometer, 166
Exosmosis, 13–14
Expenditure of water, 299–301
Exponent, hydrogen ion, 269

F

Farmer, 335
Fats, reserve, 46–48
Feeding power of the root, 250–252
Fermentation, alcoholic, 97–101
Fernandez, 92
Fertilization, 437–440
physiological basis of, 273
Fertilizers, artificial, 235–237
mineral, 236–237
natural, 235–236
Filling of grain and fungous diseases, 383
Filling of grain and soil salts, 382
Fisher, 53, 60
Fitting, 440
Fluorescence of chlorophyll, 177
Formaldehyde theory of photosynthesis, 185
Formulae, of evaporation, Dalton's, 301
of growth, 122–123
Frost resistance, methods of determining, 357–358
of plants, 355
Fruiting, 410–412
Fungus diseases, 382–387

G

Garner, 422
Gassner, 131
Gedroitz, 253–254
Gelatinization, 8
Gels, 7, 11
Geotropism, 145–150
Germination of pollen, 437–439
Germination of seeds, 113–114
Gibbs' rule, 26
Gilbert, 235
Globulins, 54
Glucoproteins, 54, 77
Glucose, 44, 56–58, 64, 70
Glucosides, 41, 59, 69
Glutathione, 108
Glutelins, 54
Grafting, 403–407
Grand period of growth, 121
Gravitational water in soil, 288
Green manure, 236
Growth, of cells, 112–118
  of cereal stem, 143–146
  and development, 409
Growth hormone, 124–127
Growth movements, 143–145
Gurvitch, 89, 128
Guttation, 284

H

Haberlandt, 127, 129
Hales, 320
Halophytes, 378–381
Harden, 99
Hardening, against drought, 365
  of winter plants, 355–358
Hardy's rule, 7
Harvey, 198, 357–353
Hauer, 373
Haworth, 39
Heat resistance of plants, 365–366
Hellriegel, 231
Hemin enzymes, Warburg's, 105
Heterotrophic plants, 223
High temperature, influence on plants, 361–363
Hill-Croft, 64
Hoagland, 30
Hopkins, 108
Hormones, 124–127, 403
Hydration, 5–28
Hydrature, 136
Hydrogen acceptors, 102
Hydrogen bacteria, 181
Hydrogen-ion concentration, influence on plants, 257, 268-271, 352
Hydrogen-ion exponent, 269
Hydrolase, 68
Hydrolysis, of fats, 47, 59
  of proteins, 50–52, 55
  of starch, 44, 56–59, 78
Hydrophytes, 366
Hydrosols, 5, 7, 10
Hydrotropism, 157
Hygroscopic water in soil, 288

I

Ice crust, 359
Iljin, 306
Imbibition water in soil, 288
Immunity, 385–386
Impermeability of protoplasm, 16–17
Infiltration, 307
Ingen-Housz, 175
Intensity, respiration, 94
  transpiration, 300
Inversion, 41, 58
Invertase, 58–59, 63
Iron bacteria, 184
Irrigation, 374
Isoelectric point, 6
Ivanov, 71, 99

J

Jodidi, 59
Johannson, 456

K

Karrer, 53
Keilin, 109

KIDD, 88
KIESEL, 34, 37
KIESSELBACH, 191
KLEBS, 412
Knight's wheel, 145
Knop's solution, 240
KÖGL, 126
KOLKUNOV, 372
KOLKWITZ, 93
KOSTYTCHEV, 67, 84, 90
KUJIPER, 92

L

LAVOISIER, 105
Law, of Dalton, 301
of diminishing return, 266
of limiting factors, 206–207
of minimum, 263
of Stefan, 190
of Zalensky, 370
Laws, 235
Laws of growth, 121–123
Leaf fall, 454–456
Leaf mosaic, 151–153
Lecithins, 26, 48
LEPESCHKIN, 27, 77
Leptohormones, 127
Leucophyll, 173
Leucoplasts, 168
LIEBIG, 263
Light, effect of, on photosynthesis, 193–197
on transpiration, 362
and growth rate, 131–135, 413
in hardening plants, 355
in seed germination, 461
in vegetation and reproduction, 423, 425–428
Light minimum, 196–197
Light plants, 193–196
Light stage, 419
Limiting factors, 206–207, 263
LINNAEUS, 433
Lipoids, 2, 10, 26–27, 48
LITVINOV, 372
LIVINGSTON, 311
Lloyd's method, 307
LOEB, 140
LOEW, 77
Long-day plants, 423, 427
Long-night plants, 427
Loss of ash elements, 257
LUNDEGÅRDH, 31–32, 207
LYSENKO, 131, 427

M

McALLISTER, 85
MALPIGHI, 320, 343
Maltase, 58, 68
MATTHAEI, 205
Maturing, of fruit, 409–412
of seeds, 442–445
MAXIMOV, 371
MAZÉ, 257
Membrane, protoplasmic, 13
Mesophytes, 366
Method, of artificial cultures, 240–244
infiltration, 307
Lloyd's, 307
Neubauer's, 262
Sach's, 119, 166
wilting, 368
Methods, of determining frost resistance, 357–358
of determining osmotic pressure, 13–16
of determining the photoperiodic reaction, 339
of determining soil fertility, 258–264
of determining suction tension, 24
of pot culture, 258–261
of studying growth, 118–123
of studying photosynthesis, 164–167
of studying respiration, 84–89
of studying stomatal movements, 306–308
of studying transpiration, 295–299
of studying water absorption by roots, 291–292
of translocation, 339
of yarovisation, 418–421

Micelles, 4, 7, 28, 30
Miller, 71
Millon's reaction, 51–52
Mimosa, 435
Mineral fertilizers, 436–437
Mineral nutrition, on natural soils, 252
of plants, 252
Mineral substances, indispensable for plants, 219
in reproduction, 438
Mitogenetic rays, 89, 128
Miyoshi, 156
Molisch, 411
Mosaic theory of permeability, 27
Mothes, 227
Motive forces of the water current, 325–327
Movements, growth, 143–145
nyctinastic, 433–435
photonastic and thermonastic, 433–434
seismonastic, 435-436
Münch, 339
Müntz, 59
Murneek, 412, 416, 425, 442

N

Nabokich, 97
Naegeli, 4-5
Nastics, 144, 433–434
Nathansohn, 27
Natural fertilizers, 235
Neljubov, 150
Neubauer, 262
Neuberg, 68
Nitragin, 234
Nitrate nitrogen, 220
Nitrification, 181–183
Nitrification bacteria, 61
Nitrogen cycle in nature, 235–236
Nitrogen fertilizers, 236–237
Nitrogen-fixing bacteria, 229–231
Nitrogen-reserve substances, 50
Nodule bacteria, 232
Nodules of leguminous plants, 231–235
Northrup, 63
Novacky, 443
Nucleoproteids, 54, 76
Nyctinastic movements, 433–435

O

Oil seeds, 29
Olsen, 270
Oparin, 65, 70, 72
Oppenheimer, 68, 105
Ordojan, 164
Organic acid, 48–50
Organic nitrogen, 220
Osmometer, 13
Osmosis, 11, 13
Osmotic concentration, 19
Osmotic pressure, 13–16
Osmotic pressure, methods, 16–24
Osterhout, 30
Overton's theory of permeability, 26, 48
Oxalic acid, 49
Oxidases, 103
Oxidation-reduction potential, 105
Oxygen acceptors, 104
Oxygenases, 104–105

P

Palladin, 48, 101
Papain, 60–61, 67
Parthenocarpy, 440
Pasteur, 97
Pavlov, 67
Pepsin, 61
Peptones, 60
Period of dormancy, 453
Periodicity of growth, 118, 131, 412
Permeability of plasma membrane, 25
Peroxidase, 104
Perrin, 4
Petri, 31
Pfeffer, 14–15, 84
Pflüger, 77
Phacophytin, 171
Phosphatase, 99

Phosphatides, 3, 36
Phosphorus cycle, 246–247
Photonasty, 433–434
Photoperiodism, 423
Photosynthesis, 161
Phototropic camera, 153
Phototropism, 150
Physiological acidity and alkalinity of salts, 224
Physiological role of cations, 247–249
Physiological self-sterility, 438
Physiologically dry soil, 268–273
Phytin, 246
Pigments, of chloroplasts, 168
  respiration, 104
Pirschle, 31
Plants of a long and short day, 423–427
Plasmodesmen, 342–343
Plasmolysis, 16–20, 25
Plastids, 167
Polarity, 402
Pollination, 437
Polypeptides, 60
Popoff, 142
Porodko, 156
Porometer, 307
Potometer, 291
Prianishnikov, 72, 74–75, 78, 257
Priéstley, 161
Prolamins, 54
Proteases, 59–60
Protection from winter desiccation, 360–361
Protective action of snow cover, 360
Protective action of sugars, 353–354
Protein composition, 2
Protein reserve, 50–54, 59, 76
Protein synthesis in the plant, 76–80
Proteinases, 61
Proteins, constitutional, 29
  enzymatic decomposition, 72–76
Protoplasm, chemical composition, 34–37

R

Rasumov, 426
Redox potential, 107
Reduction, oxidation, 106
Refractometer, 358
Regulation of transpiration, 302–303
Relation between photosynthesis and the yield, 210–211
Renner, 333
Reproduction phases, 437–440
Requirement in cold, 131
Reserve proteins, 50–54, 59, 76
Resistance, cold, 350–352
  drought, 370–372
  frost, 352
  salt, 378
  winter, 358–361
  of wood to ascent of water, 334–336
Respiration, anaerobic, 96–97
  and chemosynthesis, 181–182
Respiration enzymes, 98–100
Respiration pigments, 104
Respiratory quotient, 89–90
Reversibility of enzymatic reactions, 64–65
Rhizocaline, 403
Ringer, 140
Ringing, 321–322, 402–403
Ripening, of fruit, 409–412
  of seeds, 442–445
Robertson, 122–123
Root hairs, 289
Root pressure, 283–285
Root system of plants, 279–282
Ruhland, 26
Rule, Gibbs', 26
Rule of Hardy, 7
Rule of van't Hoff, 91–92, 204–206

S

Sabinin, 27
Sachs, 119, 121
Sachs' method, 166–167
Sachs' test, 147, 162
Salt resistance of crop plants, 382
Salt solutions, toxicity of pure, 139–142
Salts, physiologically acid and alkaline, 224

ponification of fats, 47
SAUSSURE, DE, 84
SCHANTZ, 290
SCHÖNBEIN, 103
SCHRÖDER, 192
SCHULOV, 257
SCHULZE, 74
Seeds, oily and starchy, 36–37
Seismonasty, 435–437
Selective permeability, 14, 28
Semipermeable membranes, 14–15, 25–26
SEMPER, 63
SENEBIER, 181
Shade plants, 196–197
Shedding of fruit, 441
SHIRLEY, 372
SHIVE, 141
Short-day plants, 423–425
SHULL, 21
Soil-absorbing complex, 253
Soil fertility, methods of determining, 258–264
Soil solution, 254
Sols, 4
Solution, balanced, 139–142
  Crone's, 257
  Knop's, 240
Specificity of ferments, 66–67
Stages, in the development of plants, 416–418
  of yarovisation and light, 419
STANLEY, 55
Starch, 43, 45
Starch synthesis, 162–164
Starchy and oily seeds, 36–37
STARLING, 124
Sterile cultures, 221–222
STEWARD, 31–32
Stimulation of growth, 130–161
Stomatal movements, 305–306
Stomatal regulation of transpiration, 308–310
Stratification, 460
Stroma of plastids, 168
Structure of seeds, 112–113
Succulents, 49–50, 367
Suction tension, of cells, 22–24
  methods of its determination, 24
  of roots, 282–283
Sugar content in overwintering plants, 353
Sulphur bacteria, 183–184
Sulphur cycle, 245
Sulphydryl group, 108
Superoptimal temperature, 361
Swelling of colloids, 8, 11, 93
Swelling of seeds, 20

T

Temperature and the formation of potato tubers, 426
Temperature coefficient, 91
Temperature influence on plants, 361–363
THATCHER, 444
Theory, of cohesion, 332
  of equilibrium, Donnan's, 30–31
  of oxidation, Wieland's, 102
  of permeability, Overton's, 26. 48
    Traube's, 14, 26
  of photosynthesis, 160–162
  of respiration, Palladin's, 101
    Warburg's, 104
  of stages, Lysenko's, 416–422
Thermonasty, 433
Thermotropism, 155–157
Time, presentation, 147
Toxic action, of heavy metals, 141
  of salt solutions, 139–143
Translocation, of assimilates, 337–338
  of stimulus, 433
Transpiration, 294
  cuticular and stomatal, 303–305
Transpiration balances, 299
Transpiration coefficient, 300
Transpiration formula, Dalton's, 301
Transpiration organs, 303–305
Transpirograph, 299
Transplanting, 414
Traube's theory of permeability, 14, 26
Traumatropism, 158

Tree girdle, 402–403
Tropisms, 144, 433
TUMANOV, 313, 373
Turgor, 17–23, 312, 447
Turgor pressure, 17, 22–24

U

Unavailable water reserve in the soil, 289
Urea, 69, 75–76
URSPRUNG, 24, 117, 331
USPENSKY, 272

V

Van't Hoff's rule, 91–92, 204–206
Vegetative propagation, 398
VERNADSKY, 240
VINOGRADSKY, 183, 229
VOTCHALL, 336

W

WALTER, 136–137, 371
WARBURG, 86
Warburg's theory, of photosynthesis, 187
of respiration, 104
Water balance of plants, 279–282, 311
of arid regions, 313
Water-balance influence on reproductive phase, 439
Water cultures, 240–243
Water deficit, 310–311
Water-retaining force of the soil, 287
Water supply and growth, 135–139
Wax, 48
WENT, 125–126
Wieland's theory of oxidation, 102
WILLSTÄTTER, 61–63, 174, 180
Willstätter's theory of photosynthesis, 184–187
Wilting, 311–314
Wilting coefficient, 289
Wilting methods, 368
Windscorch of grain, 365, 383
WINKLER, 405–407
Winter resistance, 355–356

X

Xanthophyll, 171–172
Xanthoprotein reaction, 51–52
Xeromorphism, 370
Xerophytes, 365–369

Y

YOKUM, 70
YOUNG, 91

Z

ZALENSKY, 369–370
ZALESSKY, 228