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PLANT PHYSIOLOGY

BY

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THIRD EDITION

WITH 70 ILLUSTRATIONS



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PREFACE TO THE THIRD EDITION

In this edition new matter will be found most extensively in the treatment of oxidation enzymes, zymase and phosphorylases (pp. 40–64); of cells as osmotic systems (pp. 75 and 78); of nitrogen metabolism (pp. 201–204); of photosynthesis (pp. 236–9 and 246–58); of respiration (pp. 268–9 and 302–6); of the metabolism of di- and tricarboxylic acids (the whole of chapter XV); and of plant growth substances (most of the pages in chapter XVII). I have transferred to chapter XVII the account of auxins which was given in the chapter on growth in the earlier editions of this book.

Because the whole of the type has been set up anew for this edition, the task of revising proofs has been arduous. It would have taken longer had I not been helped by two of my colleagues. I express my gratitude to Mr. S. L. Ranson for reading the proofs so carefully, and to Mr. J. A. Richardson for his assistance in preparing the indexes.

I am indebted to Professor T. A. Bennet-Clark and the Editors of the *New Phytologist* for permission to reproduce, in chapter XV, several of the illustrations which appeared in a review written by Professor Bennet-Clark, and to Dr. T. Mann and Dr. C. Lutwak-Mann for permission to publish figure 2, which is a photograph of a scheme constructed by them. All the other new illustrations have been made by Mr. P. Gibson.

M. THOMAS.

PREFACE TO THE FIRST EDITION

"On all great subjects, much remains to be said." This is in several senses applicable to the study of the functional processes that occur in plants, and no apology is necessary for the publication of a new book on this great subject. It is true that excellent recent and older works exist, but they are not numerous, and there is still room for several more in which the subject is treated from different standpoints and with different objects in view.

The present book has been written to assist students who wish to develop the knowledge of plant physiology that they have acquired in general courses on botany given in the higher forms at school or in the first year at a university. It is therefore hoped that it will prove useful to students of chemistry, physics, agriculture, and other subjects, who have acquired such knowledge, as well as to students who are making a special study of botany. It cannot be too strongly emphasized that there is immense scope for pioneering investigation for those who can apply in the field or in the laboratory modern knowledge of physics and chemistry to problems of plant physiology.

The range and limits of this work and the plan adopted are indicated

by the list of contents. A few additional remarks may prove helpful. The first point to note is that anyone competent to produce a complete work on the physiology of plants would require for his discussions the accommodation of several volumes, each of considerable size. I only accepted the invitation to write this book because the limits of size set —which my publishers have since generously allowed me to exceed— precluded my attempting to treat the whole of the subject. I decided to confine my attention to the analysis of the principal physiological processes that occur in green plants, and to have in view the formulation in terms of physico-chemical concepts of problems for further consideration by the reader. Even within these limits the treatment is far from complete. For instance very little attention is given to the physiology of development and none at all to the question of reproduction.

Students who already possess some knowledge of the subject should be able to read with understanding the chapters in the sequence in which they appear.[1] But it must be pointed out that as a result of the arrangement adopted it has sometimes been necessary to use for illustration in the earlier chapters facts and ideas that are more fully considered in a subsequent part of the book. There are, however, ample cross-references in the text, and the index is of fair size. Sections on such aspects of organic chemistry and physical chemistry as have a bearing on the arguments in the main body of the book have been brought together in appendices I and II. In writing these I have been forced to assume in the reader an elementary knowledge of physics and chemistry.

It is hoped that when this book has been mastered the reader will derive increased profit from his study of larger or more advanced works, monographs, reviews, and accounts and discussions of original work in current periodicals. I have not attempted to make this book a key to modern literature. Other books, such as that of Barton-Wright (*17*) in the "Recent Advances" series published by J. & A. Churchill Ltd., serve this purpose. For certain subjects, however, I have pointed out the sources from which more complete and original information may be obtained. The italicized numbers appearing after the name of an author refer the reader to the citation of a published work in the bibliography which constitutes appendix III.

My sincere thanks are offered to my sister Dr. Nesta Ferguson, who has carefully and critically read the whole of the manuscript and proofs; to Professor A. Ferguson for much valuable advice; and to Dr. R. D. Haworth of Armstrong College for the considerable help he gave me while I was writing appendix I and for reading the proofs of this appendix. All the illustrations in the book have been drawn by Mr. P. Gibson of the Department of Botany, Armstrong College.

M. THOMAS.

[1] The lecture course on which the subject-matter of this book is based actually begins with the study of biophysics as represented by chaps. I, II, and IV, taken in conjunction with appendix II. Questions of general physiology are then considered, and the course ends with the study of biochemistry as represented by chaps. II (sections A and C), III, XI, XII, XIII (section D), XIV (sections E, F, and G), and appendix I.

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behaviour of the organism during subsequent growth. Nucleated protoplasm, then, governs growth and development, and, in the reproductive phase of the life-cycle, by forming the connecting link between successive generations, serves to perpetuate the general characters of the race to which an individual belongs.

The simplest aggregation of matter in which these phenomena have been observed is protoplasm containing nuclear material. The living cell, as defined above, therefore represents one of the irreducible concepts of biology.

B. The Functional Endowments of Living Cells

By attributing to the powers resident in protoplasm such increase in size and change of substance and form as occur during cell division and differentiation, one assigns to protoplasm the power of transforming matter and energy in a specific manner. Every initial living cell is a specific metabolizing system, and every living tissue produced during growth possesses specific metabolic powers. Protoplasm in growing regions assimilates some of the food material with which it is supplied and derives energy for this essential synthesis by oxidizing another part of this food (pp. 180 and 260). Oxidations in which energy is liberated occur in all living cells, and comprise the respiratory processes of cells. Respiration is thus one of the signs of life; even dormant seeds show this sign.

Some of the respiratory energy that is not used in vital processes is liberated as heat (p. 266). Recent work suggests that electrical energy as well as chemical and heat energy may be generated inside living cells. In Osterhout's laboratory during the course of certain experiments on the marine alga Valonia, immersed in sap expressed from the large central vacuole, a persistent electromotive force of approximately 14·5 millivolts was observed across the thin protoplasmic film which lined the cell wall of this cœnocyte. No electromotive force was detected when killed cells were used. Consequently the generation of electrical energy in living cells has been described as a bioelectric phenomenon, and protoplasm as "a self-charging accumulator capable of doing work" (Gray, *99*, p. 24).

Inheritance determines not only the specific metabolic powers that are developed by living tissues, but also the relative ease with which living cells allow substances to pass in and out. Powers of specific absorption, and specific elimination appear then to be among the endowments of protoplasm (chap. IV, B).

The characters that develop during growth, and the total behaviour of organisms, are not absolutely predetermined by the factors of inheritance contained in the protoplasm. They are rather the products of the interplay between these factors and certain significant environmental factors. The environment is said to have a modifying

influence upon the development and behaviour of growing organisms (p. 343). Thus the environment provides the matter and energy for the healthy growth of green plants, and, obviously, different environments may have different nutritive values for a given race. For descriptive purposes certain of the environmental influences may be usefully grouped together as stimuli. The way is thus prepared for further analysis and for description in physico-chemical terms of the chain of reactions involved. A *stimulus* may be defined as an external or internal agent that evokes in an organism a response in which the amount of energy liberated greatly exceeds that brought into the reacting system by the inducing agent. In this book the term will be used in this limited sense, and connoting no additional properties. Thus we do not imply that a threshold value must be exceeded before a stimulus is effective, nor that stimulus reactions are, like the firing of a match, "all or none." [1]

Formative stimuli (p. 343), and those governing the orientation of plant members or inducing movement (p. 388) will be considered later. Here we note that the power and mode of response are governed by what is termed the specific irritability of living cells, which are said to perceive stimuli; changes, which lead to response, are wrought. Thus Chlamydomonas swims towards a unilateral source of light of moderate intensity, and Amœba ceases to show pseudopodial movement and becomes spherical under the influence of an electrical stimulus.

In addition to external stimuli, which cause induced responses, stimuli may be generated within living protoplasm. These will evoke autonomic responses. Such movements of Chlamydomonas and Amœba as are not directed by external agencies may be described as autonomic. Other good examples of autonomic movements that are readily observed in living cells are the circulation of protoplasm in cells near the midrib of a leaf of Elodea, in the staminal hairs of *Tradescantia sp.*, and in filaments of Nitella. The power of movement in response to internal or external stimuli is another of the signs of life, for such movement implies the presence of irritable living cells, and therefore of protoplasm.

It appears then that two identical living cells, or two twin complex organisms that have been derived from a given kind of protoplasm, may exhibit striking differences in mode of growth and general behaviour when placed under different environmental conditions. This does not mean that protoplasm becomes fundamentally changed by environmental agencies, but it does mean that protoplasm of a

[1] Went and Thimann (*328*, p. 244) consider the stimulus concept to be a "somewhat old-fashioned idea," but have not persuaded Jost (*159*) that the time has come when this and allied notions used by Pfeffer can profitably be discarded. The question of the present utility of this idea provides an interesting subject for debates.

given kind is endowed with far more extensive powers than can be revealed by keeping it continually in a single environment of fixed composition.

C. The Necessary Conditions for Protoplasmic Activity

It is obvious that constructive metabolic processes that lead to growth depend upon the presence in the environment of suitable food, or of raw material that can be transformed into food. For this transformation to be effected at a sufficient rate for the growth of green plants, light energy of wavelengths 650–700$\mu\mu$ must be incident upon the leaves. In certain plants (*e.g.*, green plants) growth (p. 351) and many associated processes (*e.g.*, the circulation of protoplasm) cease under anaerobic conditions. Such aerobic plants can procure the necessary energy from foodstuffs only in the presence of oxygen. Tissues of many aerobic plants can, however, keep alive for short periods in the absence of oxygen, and return to full activity on re-exposure to air ; but prolonged exposure to anaerobic conditions may lead to injury and death, as has often been found in attempts to prolong the lives of fruits and vegetables by storing them in pure nitrogen. It is not yet definitely established whether protoplasm suffers directly from the absence of oxygen, or whether certain of the substances produced during anaerobic metabolism (p. 287) are toxic.

Active protoplasm usually contains more than ninety per cent. water, but in certain cells (*e.g.*, those of ripening seeds), this amount may be reduced to well below ten per cent. without injury. General activity is, however, greatly diminished, and what is termed a state of dormancy may result. Full activity may be regained after the air-dry cells have imbibed water and regained turgor.

Protoplasmic activity is considerably affected by changes in the temperature of the surroundings. Instructive experiments may be performed on the effects of temperature on the rates (*a*) of circulation of protoplasm in the cells of Elodea, (*b*) of growth of bacteria on slopes of nutrient agar, and (*c*) of exodiffusion of anthocyanin from the cells of red beet—three phenomena depending upon protoplasmic activity —and it will be found that protoplasm maintains its powers unimpaired only between certain temperatures. Thus prolonged exposure to temperatures greater than 40° C. causes irreparable injury. The higher the temperature the more rapidly is protoplasm killed ; 50° C. has often been cited as approximately the death temperature of protoplasm, but it is now realized that the effect of temperature cannot be dissociated from the duration of exposure (p. 350), and one may state that, as a rule, protoplasmic activity can only be maintained at temperatures between 0° C. and 40° C.[1] A rise of tempera-

[1] See Pfeffer (*228*), vol. II, p. 57, for data for different plants, including thermophile bacteria and certain algæ that can live in hot springs.

ture augments all forms of activity until a temperature injurious to protoplasm is reached, when activity diminishes.

Low temperatures, but above 0° C., may have obscure effects upon protoplasmic activity, some of which may lead to injury and death. For example, no explanation can as yet be given of the facts (*a*) that a certain minimum temperature, which varies from plant to plant, is necessary for growth, and (*b*) that certain varieties of apple turn brown prematurely when stored at temperatures lower than 5° C. At temperatures below 0° C., living cells may suffer from frost injury, especially if a period of severe frost is followed by a rapid thaw. For instance, exodiffusion of anthocyanin takes place rapidly from the cells of a red beetroot which is subjected for a few hours to the temperature of a salt-ice freezing mixture, and is subsequently transferred to a temperature of 20° C. It should be noted that the freezing point of cell sap is lower than that of water, and will depend upon the concentrations of solute molecules dissolved in the sap.

The resistance of protoplasm to the injurious effects of low and high temperatures increases as the water content is reduced. Thus dry bacterial spores have been kept at the temperature of liquid air (— 191·5° C.) without loss of vital powers, and temperatures greater than 100° C. are often necessary to kill such spores. A simple illustrative experiment with the higher plants is to compare the effect, on their subsequent germination capacity, of exposing dry and swollen seeds to low and high temperatures (*cf.* p. 350).

Experiments on seed germination (p. 350) and on the rate of exodiffusion of the sap from cells of red beetroot (pp. 84 and 86) show that protoplasmic endowments are maintained within only a narrow range of hydrion concentration, and only when there is a suitable balance between monovalent and divalent ions in environmental solutions. The osmotic pressure of the environmental solution must not be higher than that of cell sap (*cf.* water requirement, mentioned above). Implicit in the terms of narcotic or toxic substance is the fact that protoplasmic activity may be retarded or temporarily inhibited, or that protoplasm may be killed by certain chemical substances which may be present in the environment. Thus it has been suggested that the presence of carbon dioxide in relatively high concentrations in the soil may narcotize certain seeds, and so impose a state of dormancy (p. 155): and it has long been known that certain metallic ions, when present in sufficient concentration, can kill protoplasm; hence the efficiency as fungicides of spraying mixtures containing copper.

Finally, we note that the presence of such stimuli as have a formative influence on the development of essential organs (*e.g.*, for green plants light from the blue-violet end of the spectrum) is also a necessary condition for the display of the full powers of growing living cells.

D. Visible Subcellular Functional Units

The study of function requires that attention be paid to both agent and action, viz., for vital function, functional structural unit, and physiological process. It should be noted that whereas we regard the cell as the unit of structure, and must allow that only nucleated protoplasm can grow and multiply, the structural units which actuate the individual physiological processes may be of subcellular dimensions.

It is easier to investigate process, and describe what is found in suitable terms, than to attempt the elucidation of the structure and mode of action of minute protoplasmic machines. Nevertheless, analysis of protoplasm has been attempted, and proceeds in several ways. The observation and description of what may be discerned with the aid of the microscope in living cells at rest and in activity is included in the subject-matter of cytology. In books on this subject (*e.g.*, Sharp, *264*) will be found full descriptions of the structural heterogeneity of typical living plant cells—of the cytoplasm and such visible structures as plastids and mitochondria, and of the nucleus with its constituent parts. The structure and mode of action of certain of these microscopically visible subcellular units of function (*e.g.*, chloroplasts) will be considered in later chapters. It should be noted that genetics is a branch of physiology, and the chromosome theory of heredity is one which assigns to subcellular units within chromosomes functions of carrying the factors of inheritance from parents to offspring and of governing the modes of interplay between organism and environment that come into action during growth and development.

But it is usual, nowadays, owing to the rapidity with which it has developed, to study genetics as a separate subject, and a good choice of books is available (Babcock and Clausen, *9*; Punnett, *240*; Sansome and Philp, *259*).

Another way of analysing protoplasm and the parts recognized by cytologists, viz., the physico-chemical way, comes within the province of physiology, but we have no space to do more than indicate some of the problems. The aim of physiologists, who approach the concrete object, living cell, with a view to analysis, is to narrow the gap between the biological concept of protoplasm and the physico-chemical concepts of matter and energy. Those generalizations which resume the properties of inanimate objects are used to describe the properties of the parts of living objects. Attempts are thus made to describe the workings of whole cells in terms of the co-ordinated physico-chemical activities of their parts.

CHAPTER II

THE PHYSICO-CHEMICAL HETEROGENEITY OF PROTOPLASM

A. Gross Chemical Analysis

THE formulation of biological problems in physico-chemical terms requires a knowledge of the chemical composition and the physical state of the functioning units in cells. Gross chemical analysis of protoplasm collected from various sources and killed in various ways has provided valuable information, which has led to microchemical investigations on the distribution of substances in the microscopically visible structures in living cells (section C), and to important arguments concerning the physical state of cell components (section D). For example, the plasmodia of Myxomycetes, which consist of nucleated protoplasm,[1] together with engulfed food-stuffs and metabolic products of diverse functional value, have on several occasions been analysed. The results of an analysis by Lepeschkin are recorded below.

Analysis of the plasmodium of a Myxomycete (probably *Fuligo varians*)

	Per cent dry weight.[2]
A. Organic substances.	
(i.) Water soluble.	
Monosaccharides	14·2
Proteins	2·2
Amino acids, purines, asparagine, etc.	24·3
(ii.) Insoluble.	
Nucleoprotein	32·2
Free nucleic acid	2·5
Globulin	0·5
Lipoprotein	4·8
Neutral fats	6·8
Phytosterol	3·2
Phosphatides	1·3
Polysaccharides, pigments, resins, etc.	3·5
B. Mineral matter	4·4

It is assumed that the insoluble organic substances, other than those in the polysaccharide group, form the basis of the executive structural units in protoplasm, since they have all been detected in a wide range of plant cells and animal cells. It should be noted that these organic compounds are always associated with mineral matter

[1] The idea of a skeletal "living machine," *i.e.*, protoplasm proper, is a pure abstraction: what one tries to do is mentally to separate executive structural units in cells from the "raw material" and "fuel" that they assimilate or assume during growth.

[2] The percentage of water in the living protoplasm was 82·6.

in solution, and the presence of free ions of potassium, calcium, magnesium, iron, chlorine sulphate, phosphate and, possibly, of traces of manganese, copper, and zinc, may be essential for the activity of protoplasm.

Other substances, which do not appear in such an analysis table, are probably invariably present, and form an essential part of the working mechanism of living cells. The elucidation of the chemical nature of enzymes, coenzymes, activators, and other spare parts (see p. 381), adds to our knowledge of such substances. Thus hæmochromogens (*e.g.*, cytochrome), catechol compounds, certain nucleotides, ascorbic acid, and the tripeptide glutathione, may be essential components of the oxidation mechanisms that actuate vital processes. In green cells, the chloroplast pigments might be included among the executive substances, seeing that they are essential for photosynthesis. It should be noted that growth implies the synthesis by plants of the essential components of protoplasm (p. 341). In order to define problems of metabolism it is evidently of first importance to have some understanding of the chemical composition of these components. All the substances mentioned in this section are considered in Appendix I.

It is noteworthy that there is always a preponderance of hydrogen ions over hydroxyl ions in plant sap. Indeed, there is some evidence that the *pH* often fluctuates between 4 and 6. The isoelectric points of many proteins also lie between these *pH* values. Cell sap is usually on the acid side of the iso-electric points, but, in spite of the presence of buffer-substances, metabolism may bring about fluctuations in the *pH* of a given cell, and thereby induce changes in the physical state of cell proteins, such as would affect the physiological behaviour of the whole cell (Small, *270*).

We may sum up by stating that chemical analysis indicates that the protoplasm as a whole in any plant cell appears to be constructed from certain well-defined classes of organic compounds in association with acidic aqueous solutions of electrolytes.

B. The Physico-Chemical Heterogeneity of Protoplasm in relation to Varietal Differences

The chemical substances found on analysing killed protoplasm were at one time regarded as decomposition products of a single large living molecule, which was referred to as a biogen (for a critical account of this view see Hopkins, *140*). The modern view is that many of these substances occur as free molecules in protoplasm, and that protoplasm is therefore chemically heterogeneous. Nevertheless, it is probable that some separation of the molecular components of executive functional units occurs during chemical analysis. These units may be built up of chemical compounds more complex than those indicated by the results of analysis, or of distinct molecules which exist in some essential physical association within the living protoplasm. It was Nägeli who first suggested that

certain of the molecules might come together under physical (in contradistinction to chemical) forces to produce definite structural units, which he named micellæ. We may picture a micella as a unit composed of two or more molecules of the same compound or of different compounds, the molecules retaining their identity within the whole physical aggregate. Furthermore, ions may be adsorbed on the surface of a micella, and play a part in its functional activity. Molecules and micellæ may remain free, or adhere under physical forces to produce fibrils or other quasi-crystalline structures. It is possible that X-ray analysis may in the future throw light on what is at present an obscure problem.

Buildings are constructed according to plan from bricks and mortar; machines, in fulfilment of design, from spare parts; protoplasm, then, one may imagine, grows by the production and coming together of molecules and micellæ in a fashion that is governed by the inheritance of the race to which the protoplasm belongs. It is a highly significant fact that the number of different living structures that might be constructed from the classes of substances found in protoplasm greatly exceeds the number of races of living organisms which now exist, or have existed in the past and are now extinct. For instance, the protein group comprises an immense number of distinct individuals (p. 448), and certain delicate precipitation tests have indicated that differences may be detected in the proteins of two races of the same species. Racial differences must be attributed to structural design as well as to chemical differences in the building material. There will be variation in ultramicroscopical structures as well as in those structures in the nucleus and cytoplasm which can be studied with the microscope. It is clear that an almost infinite number of distinct structural forms might [1] be constructed from the large number of molecules present even in the smallest living cell, by using for each constructive operation different numbers of molecules and putting together in different ways those selected for use. For example, Haldane (*111*, p. 178) has calculated that a single yeast cell may contain as many as 150,000 molecules of saccharase (invertase). This enzyme is only one of the many enzymic and other components of the protoplasm of yeast. One may sum up by stating that the term protoplasm, when used in a physico-chemical sense, connotes a kind of material, infinitely variable, but always built on a similar general plan from molecules belonging to a limited number of well-defined classes of compounds. All living forms possess a certain community of structure as well as of faculty, but each form is in some respect *sui generis* in point of structure, and, consequently, of functional power (*cf.* older views as expressed by Huxley, *146*).

C. Distribution of Substances in Protoplasm

The inorganic constituents of cytoplasm and nuclei. Microchemical tests have been used to study the distribution of certain inorganic constituents in protoplasm. Thus the presence of iron in

[1] *I.e.*, did man possess such powers of manipulation as would permit him to work with individual molecules as a builder works with bricks. In passing we may note here that since man does not possess such ultra-delicate powers, it would be idle for him to attempt the synthesis of any given form of protoplasm, man's or amœba's. It is conceivable, however, that molecules might by chance come together under natural forces under experimental conditions, so as to yield structural forms capable of growth and multiplication. It is highly improbable, however, that such forms would exhibit kinship to existing forms of protoplasm, the products of natural causation through the ages.

cytoplasm and nuclei has been demonstrated by the use of potassium ferrocyanide and hæmatoxylin solutions, and that of phosphates by the well-known ammonium molybdate test. Potassium ions have been detected on the surface of the chloroplast of Spirogyra by precipitating the metal as potassium cobalti-nitrite. James and Penston (*157*) detected this element in the cytoplasm of the meristematic cells of the stem, root and tuber sprouts of potato, and also in green cells. The greatest precipitation with cobalt hexanitrite occurred on the surfaces of the nuclei and plastids (including the chloroplasts), but no evidence was obtained of the presence of potassium inside these structures. Neish (*208*) isolated and analysed chloroplasts from clover leaves and found that the concentration of ammonium, and possibly of other ions, was higher, and that of calcium, magnesium, manganese, sodium, and chlorine, was lower in the chloroplasts than in the rest of the cell. He states that potassium was probably absent from the chloroplasts, thus agreeing with James and Penston. Such unequal distribution of ions, if accompanied by unequal distribution of electrical charges, might govern the development of bioelectrical potentials in the protoplasm (p. 3).

The organic compounds in cytoplasm. Tests with Millon's and other protein reagents prove that proteins form the basis of cytoplasmic structure. Certain chemical reactions suggest that mitochondria contain lipoid substances, either free or combined as lipoprotein. For instance, they dissolve in ether, alcohol, chloroform, and other solvents for lipoids, unless protoplasm has previously been treated with chromium or certain other fixatives; and they occasionally react with osmic acid, a reagent which darkens substances containing unsaturated fatty-acids. Moreover, their smooth even outlines recall the so-called myelin figures seen when water is added to lecithin. There must be a whole host of water soluble and adsorbed organic substances in protoplasm, such as the coenzymes mentioned on p. 9. There are grounds for hoping that with the development of spectrophotometric methods some of these substances will be detected *in situ*. Evidence has already been obtained that ribo-nucleic acid is present in the cytoplasm of all cells, plant and animal (see p. 14).

In recent years methods have been developed for separating certain cytoplasmic components from nuclear, cell wall, and vacuolar components of the cells of green leaves (see *e.g.*, Chibnall and others, *59*, Mencke, *199*). Chibnall treated cells twice with the same aqueous ether in order to increase their permeability, and then with frequent washing squeezed out under great pressure the water-soluble crystalloidal substances, the cell walls acting as ultra-filters. He ground the residue, dispersed it in water, and separated fractions by filtration through silk gauze and paper pulp. Mencke used a method involving fractional centrifuging, fractional precipitation with ammonium

sulphate, and fractional heat coagulation, of green aqueous extracts of crushed leaves.

Chibnall analysed and described the properties of proteins that occur, so he maintained, in the cytoplasm of spinach and certain grasses (*e.g.*, cocksfoot). He also analysed the ether-soluble substances present in the whole cytoplasm (*i.e.*, including the chloroplasts) of the leaf cells of cabbage, finding in addition to pigments, fats, and sterols, considerable fractions of saturated and unsaturated unsaponifiable substances. Among the saturated substances were higher paraffins (*e.g.*, nonacosane), and alcohols and acids of high molecular weight.

Some modern views about the composition and structure of chloroplasts are discussed on p. 227 *et seq.* Mencke succeeded in isolating chloroplasts from spinach leaves, and compared their composition with that of the rest of the cytoplasm. In terms of dry weight over ninety per cent. of the cytoplasm was protein and less than one per cent. lipoid, whereas the chloroplasts contained more than thirty per cent. lipoid, and only about fifty per cent. protein. In passing, we note that Mencke also detected ribonucleic acid in chloroplasts : its presence elsewhere in cytoplasm is not excluded. Neish (*208*) isolated chloroplasts from clover leaves. He, like Mencke, found that they were relatively poor in protein and rich in lipoid. It should be noted that lipoids contain phosphorus in organic combination. He made several additional observations about the non-pigmented constituents of chloroplasts. Thus he detected in them copper and iron in organic combination. This finding suggests the presence in chloroplasts of oxidizing enzymes. Catalase activity was relatively high. Hill and Lehmann (*131*) measured the iron content of the chloroplasts of Claytonia and found that they contained four times as much iron as they would be expected to have were the iron equally distributed throughout the leaves. (It is convenient to note here that Dam *et al.* (*69*) found vitamin K_1 (2-methyl-3-phytyl-1, 4-naphthoquinone) in all the cells containing chloroplasts which they examined.) Clearly these new methods of mechanical fragmentation of protoplasm, when combined with chemical analysis and the determination of enzyme and coenzyme constituents, promise to provide information not only concerning the composition of recognized structures in cytoplasm, but also concerning the metabolic activities of these structures (see p. 235). For example, Hill (*129*) has obtained measurable amounts of oxygen by illuminating, in the presence either of leaf extracts or of ferric potassium oxalate and in the absence of carbon dioxide, chloroplasts isolated by grinding leaves in isotonic sucrose, each of which he terms "a subcellular system containing chlorophyll" (*cf.* p. 7). Evidence has thus been secured that in chloroplasts the chemical systems that

take up and use carbon dioxide may possibly be distinct from those which set free oxygen (see p. 236).

The organic compounds in resting and dividing nuclei. The presence of protein in nuclei can readily be demonstrated with Millon's and other reagents. Macrochemical methods indicate that some of the protein occurs in association with nucleic acid as nucleoprotein. Thus this alkali-soluble conjugate-protein has been found in relative abundance in animal cells (*e.g.*, sperm heads of fish) and in plant cells (*e.g.*, yeast and wheat embryos) in which the ratio of the volume of nucleus to the volume of cell is high.

Nucleoproteins differ from ordinary proteins in not being digested by gastric juice (pepsin in the presence of decinormal hydrochloric acid). They are, however, soluble in alkalies and sodium phosphate, and are digested by pancreatic juice (trypsin in alkaline solution). Towards the end of last century Zacharias, by microchemical tests, showed that the material in the nuclei of the epidermal cells of *Tradescantia virginica*, *Ranunculus lingua*, and certain other species, have the properties of nucleoprotein (then known as nuclein) towards gastric and pancreatic juices, alkalies and sodium phosphate. He reported that in the cytoplasm there was yet another protein, insoluble in gastric juice. This he called plastin. He stated that the other proteins in cytoplasm and in nucleoli were digested by gastric juice. But Wager in 1904 asserted that he found proteins insoluble in gastric juice, and containing phosphorus, in the nucleoli of Phaseolus cells. Similar experiments performed by other workers appear to have established that the chromatin material of resting nuclei, and of chromosomes of dividing nuclei, consists for the most part of nucleoprotein. Thus, Earl (*79*) made serial sections of the root tips of beans, treated the fixed protoplasm with one per cent. pepsin in decinormal hydrochloric acid, and by staining showed that chromatin was still present in resting nuclei, and that the chromosomes in dividing nuclei had not been digested. He found, however, that the spindle fibres had been digested, and concluded that they contained simple proteins as the basis of structure. Furthermore, he inferred that, had the chromatin been a simple protein, it also would have been digested.

Shinke and Shigenaga (*265*) have confirmed the fact that all the well-defined structures in living cells contain protein. In addition to making use of solution tests and staining tests for nucleoproteins, they succeeded in getting remarkably clear results by using the test elaborated by Feulgen in 1923 for detecting thymus nucleic acid,[1]

[1] Fresh material, or material that had been fixed in acetic acid and mercuric chloride, is hydrolysed with normal hydrochloric acid at 60° C. for three to five minutes. After washing in water the material is immersed in Schiff's aldehyde reagent (fuchsin containing free sulphurous acid) for one to three hours. If thymus nucleic acid is present, a red or violet colour develops.

which is now known as desoxyribose nucleic acid (see p.443). By means of this test they showed, for several plants, that this nucleic acid is present in the nuclear reticulum of resting cells, and in the chromosomes of dividing cells, but failed to detect it in nuclear sap, nucleoli, or cytoplasm. But they found that Feulgen's test always gave negative results with the nuclei of Spirogyra cells, and suggested that these nuclei contain some nucleic acid other than thymus nucleic acid. They favoured the view that nucleic acid occurs combined in nucleoprotein, because the chromosomes dissolved in alkaline solutions. Furthermore, Shinke had previously found that the optimum *p*H for the precipitation of substances in the reticulum and chromosomes was nearly the same as the isoelectric points of certain nucleoproteins. According to Mirksy (*203*) it is now proved that desoxyribose nucleic acid occurs in the cell nucleus, and that ribose nucleic acid is found mainly in the cytoplasm, and to a much less extent in the nucleolus. Davidson and Waymouth (*72*) support this conclusion.

Great interest attaches to the composition of the substances from which chromosomes are built. Recent observations indicate that chromosomes possess a spiral structure. Shinke and Shigenaga found that in the metaphase of the heterotype division of the pollen mother cells of *Tradescantia reflexa*, the thymus nucleic acid reaction was exclusively given by the spiral part, and that the matrix contained lipoid substances as well as proteins. They detected the presence of lipoids by observing the effects of lipoid solvents (*e.g.*, chloroform, benzene, and amyl alcohol) and of lipoid stains (*e.g.*, Sudan III, and chlorophyll tincture) on nuclear and cytoplasmic structures. With the lipoid stains they succeeded in staining the nuclear reticulum, chromosomes, nucleolus, spindle fibres, and parts of the cytoplasm. They also found that lipoid solvents dissolved nuclei and chromosomes, and, by applying Feulgen's test, showed that nucleic acid was thus set free and became disseminated throughout the cell. There appears, therefore, to be a variety of evidence that chromosomes, like the rest of the cell, are chemically heterogeneous.

In the next chapter the recent work on the chemistry of enzymes will be considered. We note here, however, that in bringing about some enzymic changes (*e.g.*, phosphorylation, some oxido-reductions) a coenzyme associates with a proteinaceous apoenzyme to constitute the holoenzyme. The suggestive fact has been established that at least three of these coenzymes (viz., alloxazine-nucleotide, pyridine-nucleotide, and adenylic acid) have affinities to substances that occur in chromatin. Such discoveries may lead to an understanding of some of the functions of nuclear substances in the control of metabolism.

D. The Physical Properties of Protoplasm

Protoplasm as a heterogeneous liquid system. Protoplasm

usually contains more than ninety per cent. water, and in active cells shows many of the physical properties of liquids. Without question one must regard protoplasm as liquid when it allows Brownian movement of suspended particles or is capable of flowing in a cell. Moreover, it has been observed that when certain cells are ruptured the extruded protoplasm is miscible with water, and that no phase boundaries are formed when water is injected into whole living cells.

We may directly infer from our knowledge of its chemical composition that protoplasm in the liquid state, although optically homogeneous, must actually be " an extraordinary complex heterogeneous system of numerous phases and components " (Bayliss, *20*). It has the properties of a complex hydrosol with disperse phases composed of molecules and molecular aggregates (micellæ) of proteins, lipoids, etc., and a continuous phase of a crystalloid solution containing many different solutes, both electrolytes and non-electrolytes.

It has for many years been generally agreed that vital phenomena are in some way bound up with the properties of matter in the colloid state. More recently it has been suggested that mechanical and electrical adsorption (p. 458), and the forces determining the orientation of molecules at phase boundaries (p. 459), may cause order to develop in what might otherwise be a chaotic mixture of solutes. Professor Frey-Wyssling of Zurich has made a special study of what he terms the submicroscopic morphology of protoplasm and its derivatives. His suggestive ideas concerning the modes of association of proteins, lipoids, and other components of protoplasm, have been summarized (Clarke, *62*). He visualizes a colloid system in which there exists as solid disperse phase a micellar framework of filamentous molecules of proteins, and in the intermicellar spaces a continuous phase of water, inorganic salts, and other soluble substances. The proteins are supposed to be composed of long polypeptide chains, bearing side-chains, which may be hydrophilic, lipophilic, acidic, or basic. He pictures other components (*e.g.*, phosphatides, and lipoids) as associated with these side-chains, and orientated according to their chemical affinities (*e.g.*, acidic and basic side chains will react with one another), or to the polar properties of the molecules concerned (*e.g.*, lipophilic groups associate, hydrophilic groups will be directed towards aqueous phases). Either this or any other view of the ultramicroscopic structure of protoplasm will suggest that, as a result of the colloid dispersion of cell components, the surface area between the dispersed and continuous phases in protoplasm must be very great in proportion to the volume of the protoplasm as a whole (*cf.* p. 457). In a single cell there are many millions of molecules of organic and inorganic solutes in solution, and their fate will be largely determined by the physical and chemical forces resident in these extensive and highly differentiated internal surfaces (see chap. III).

Changes in the viscosity of protoplasm. It is well known that the consistency of certain emulsoid sols is not an unvarying property, but alters with the temperature, and with the composition of the continuous phase. On lowering the temperature, or increasing the concentration, such sols may set to form plastic or rigid gels (p. 455), which may be reconverted into sols by warming or dilution. Reversible transformations of sol and gel have also been observed in plant protoplasm. Thus Seifriz found that the application of pressure with a glass needle, so as to close a hypha of Rhizopus, caused protoplasm in the sol state to be converted into a rigid gel. Scarth has observed that the protoplasmic threads which traverse the vacuole of a mesocarp cell of Snowberry sometimes behave as inextensible threads, and snap when tensions are applied to them. At other times, however, these threads are the seat of active streaming movements, and may be pulled into threads which possess elasticity. Clearly the physical state of the emulsoid systems in cells is highly variable. These systems may be in the form of plasmasols or plasmagels, and the latter may be rigid or elastic. It is not yet clear what biological significance may be attached to these changes of state from sol to gel, for the general activity of cells does not appear to be impaired by the gelation of plasmasols. Stiles (*289*) has severely criticized the view that viscosity may govern the velocity of certain metabolic changes by affecting the rates of diffusion of the reactive solutes. It appears that solutes diffuse nearly as rapidly in gels as in sols. One must admit, however, that we do not yet know whether convection currents or other forms of mass movement of particles play a part in distributing metabolites in cells other than sieve tubes, tracheæ, and latex vessels. If they do, it is conceivable that viscosity may at times have a considerable indirect effect on the rate of metabolic events.

Furthermore, the viscosity of protoplasm even when it remains in the liquid state as plasmasol is not of fixed magnitude. The viscosity of plant protoplasm has been measured in a number of ways. For example, determinations have been made by measuring the rate of protoplasmic streaming, the rate of fall of starch grains, the rate of movement in an electric field of nickel particles introduced into cells, and the displacement of particles showing Brownian movement. For many years it was supposed that plasmasol is always highly viscous, even more viscous than castor oil, which is one thousand times as viscous as water. But Heilbronn concluded from his measurements of the velocity of fall of starch grains within cells of a section of *Vicia faba*, that protoplasm may be less than eight times as viscous as water. In general, one may state that the viscosity may range from such low values to the infinite viscosity of plasmagels. And it must be realized that diverse states may coexist in a single cell.

Alterations in the viscosity of a given plasmasol may be effected

in a variety of ways. The influence of temperature has been carefully studied, and more than one type of alteration has been observed. Thus the behaviour of certain forms of protoplasm resembles that of non-living systems, in that the viscosity steadily diminishes with increasing temperature. Baas Becking's measurements of the viscosity of the protoplasm of Spirogyra illustrate another type. The viscosity increased only slightly between 10° C. and 20° C. At higher temperatures it rose sharply to a maximum at 27° C., and then fell sharply. At temperatures just above 50° C., the death temperature, protoplasm quickly coagulates, and, of course, the viscosity rises. Doubtless coagulation must be attributed to the effect of heat on one or more of the proteins in protoplasm. It is important to note, however, that no protein is known which will coagulate in solution at so low a temperature as 50° C. This again illustrates the heterogeneity of protoplasm. The presence of *all* the different components must be considered in attempting to account for the physico-chemical behaviour of protoplasm. The experiments of Heilbrunn (*126*) indicate that changes in the state of the fats and lipoids in cells play an important part in determining the behaviour of protoplasm to increasing temperatures, and that the presence of metallic ions, particularly those of calcium, may lower the coagulation temperature of certain proteins.

There appears to be good evidence that whereas protoplasm coagulates under the influence of alcohol, ether, chloroform, and other fat solvents in high concentrations, such fat solvents in low concentration may cause a decrease in viscosity. Other external factors which are known to affect the behaviour of plant cells (chap. IV, B) and also to affect viscosity are the hydrion concentration, and the balance between monovalent and divalent ions. These same factors may also affect the viscosity of protein hydrosols. It is probable that light will be thrown on many obscure problems of cell physiology by the use of physical methods in experimental cytology (see Gray, *99*).

Plasmatic membranes. It has for many years been widely held that every protoplasmic mass is enveloped by a definite structural unit to which the name plasmatic membrane has been given. Thus it has been maintained that naked protoplasmic masses (*e.g.*, Amœba, zoospores, motile gametes) would become colloidally dispersed in water were not each mass surrounded by some sort of pellicle; and it has been suggested that surface-tension films, such as might be produced by mechanical adsorption at phase-boundaries (p. 458), would suffice for the maintenance of the integrity of each mass. Such surface-tension films would be invisible even under the highest powers of the microscope. From certain electrical measurements it has been calculated that the thickness might be as low as 3×10^{-7} cms,

which is about the length of a single protein molecule. It is possible that such a plasmatic membrane would be a monomolecular film.

Physical arguments, in addition to supporting the view that plasmatic membranes exist, throw light on their composition. Thus it is probable that proteins, lipoids, and any other components of the complex protoplasmic hydrosol that reduce interfacial tensions, would be adsorbed at interfaces. Certain experimental facts (see, *e.g.*, p. 83) can be explained if it is assumed that surface-tension films formed as a result of adsorption are heterogeneous structures composed of cohering aqueous and non-aqueous phases—a sort of mosaic of lipoid substances with no affinity for water, and a proteinaceous hydrosol or hydrogel.

It should be noted that for each vacuolated plant cell there would be at least two films, viz., the outer plasmatic membrane between protoplasm and the wet cell wall, and the inner plasmatic membrane between protoplasm and the vacuolar solution. Since the conditions at a phase boundary will be governed by the composition of both phases, and since the composition of vacuolar solutions usually differs from that of the solution held by imbibition in cell walls, one would expect the composition and properties of inner and outer plasmatic membranes to show differences. Osterhout (*218*) has adduced experimental evidence in support of the view that the properties of the two pellicles may differ. He found that magnesium was absent from the cell sap of the marine alga Valonia, and inferred that the inner plasmatic membrane of this cœnocyte is impermeable to magnesium ions. The other plasmatic membrane, he argued, must permit the passage of magnesium ions, for, were it impermeable, chlorophyll, in each molecule of which there is one atom of magnesium (p. 441), could not be produced. Bennet-Clark and Bexon (*24*) have made the observation, which is also significant in other directions (p. 78), that malate supplied from the outside, in concentrations very much lower than those prevalent in the vacuolar sap, to cut discs of beet immersed in liquid accelerates the rate of respiration of the discs. One of their suggestions is that those respiratory enzymes whose activity is affected by malic acid may be located on the outside surface of the protoplast; accordingly a lower concentration at this surface may be more effective than a higher one on the inside surface. In this suggestion the physico-chemical heterogeneity of protoplasm (Section B) is emphasized. So it is in their alternative suggestion, which is also in line with Osterhout's hypothesis. In this they postulate "an external plasmatic membrane having greater permeability than the vacuolar boundary membrane coupled with location of respiratory enzymes within the protoplast." This might allow malic acid even in low concentrations to reach the enzymes from the outside more rapidly than it can from the higher concentration in the vacuole.

It must not be supposed that the plasmatic membranes of a given cell possess a fixed composition and unvarying properties. Rather they should be considered as mutable films in which changes of composition or of distribution of phases in the mosaic, with consequent changes of properties, may be the sequel to the occurrence of events in the cell, or to alterations in the environment. Spontaneous and induced changes in the permeability of protoplasm will be considered later (p. 84). Here we note that plasmatic membranes may play an important part in determining the osmotic

properties of living cells. Consequently, considerable interest attaches to such experimental facts and theoretical arguments as may throw light on the mutability of plasmatic membranes. For example, dilute solutions of lipoid solvents (alcohol, ether, chloroform, benzene, etc.) may occasion alterations in plasmatic membranes, and, accordingly, affect their physiological properties. In addition to exercising a solvent action, they act by lowering interfacial tensions between protoplasm and the solution in which the cell is immersed. Then, changes in the hydrion concentration of cell sap, or of the solution which wets a cell wall, may affect the physical state of proteins in the aqueous phase of the plasmatic membrane, and, consequently, the permeability of the whole unit (*cf.* p. 85). We point out elsewhere (p. 85) that the properties of living cells are affected by the ionic composition of the solutions which bathe the protoplasts. We note here that salts of sodium (or potassium) and calcium may have different effects on a complex hydrosol containing lipoid. Clowes prepared a stable emulsion of oil in water by shaking olive oil with water and a sodium salt, and of water in oil when he used a calcium salt. Phase reversals of aqueous and non-aqueous phases may thus be induced by a change of balance between monovalent and divalent ions in the environment of protoplasts. Other physico-chemical effects, such as the dispersing effect on proteins of salts of monovalent metals and the tendency shown by calcium to combine with constituents of the plasmatic membranes, may affect the behaviour of plasmatic membranes under certain conditions. And it must be remembered that proteinaceous hydrosol or hydrogel systems undergo spontaneous changes on keeping (pp. 449, 456); so to speak, they age. The idea that plasmatic membranes exist arose from physico-chemical arguments. As physico-chemical knowledge has grown, *pari passu* the idea has been elaborated. It must be remembered, however, that with the further growth of knowledge the idea may prove no longer tenable, and may be replaced by a new concept of the physical structure of cytoplasm.

CHAPTER III

PROTOPLASM AS A CHEMICALLY ACTIVE SYSTEM

A. Enzymes as Units of Chemical Activity

THEODOR SCHWANN (1889) coined the adjective *metabolic* to describe the chemical changes that accompany or govern vital processes. Shortly afterwards it became clear that metabolism is a property of what we now term protoplasm, and the view has since developed that the whole protoplasm in a given living cell possesses extensive chemically active surfaces on which highly diverse metabolic events occur in an organized manner. It is supposed that there is division of labour among different portions of protoplasm in a cell, and that the harmonious behaviour of the whole results from co-ordinated specific activities of these subcellular functional units.

Specific chemical powers have been attributed to a few visible structures (*e.g.*, plastids) in specialized cells, but, from the outset, analysis of protoplasm as a chemically active system has proceeded not along cytological but along biochemical lines. Even before fundamental ideas concerning the chemical powers of living cells were put forward, it was known that living organisms contained catalysts. As an outcome of later work these catalysts were termed *enzymes*, and biochemical analysis has proceeded intensively with a view to separating as many individual enzymes as possible from the protoplasm of living cells, plant and animal, and to studying the powers and properties of every single enzyme, as displayed *in vitro* under defined conditions.[1] Enzymes are regarded as subcellular functional units of chemical activity. It is supposed that they possess the same powers and properties when they form part of the living protoplasm, *i.e.*, are functioning *in vivo*, and that knowledge gained by studying their behaviour *in vitro* may therefore be applied to describe more precisely the metabolic events that occur in whole cells.

B. The Separation and Concentration of Enzymes

Most enzymes are soluble in water, and certain enzymes (*e.g.*, the exo-enzymes of saprophytes) readily diffuse out of living cells; but many are tenaciously held by substances in cells, or protected by membranes, and cannot be separated in appreciable amounts until cells have been subjected to preparative treatment. Extraction by

[1] Sumner and Somers (*298*) have written an admirable account of the general properties of enzymes, and of the nature and activity of individual enzymes. I. the volumes of the "Annual Review of Biochemistry" recent advances are summarized, and valuable discussions will be found in the volumes so far published of "Advances in Enzymology."

water, dilute glycerine (in which solvent enzymes appear to be more soluble and stable than in water), or dilute acid or alkali, is facilitated if cells have previously been dried, frozen and allowed to thaw, plasmolyzed, or allowed to autolyze, so as to disintegrate cell structures and render membranes more permeable. For example, emulsin readily passes out from well-ground dry kernels of sweet almond when these are placed in dilute alkali; and a good yield of invertase can be obtained from yeast that has been allowed to autolyze in the presence of toluene or chloroform for a few days.

Very special interest attaches to the first separation of zymase from yeast, for alcoholic fermentations had previously been attributed to the activity of whole cells. Buchner (1897) destroyed cell structure by grinding yeast with a fine siliceous earth called kieselguhr, and then, by subjecting the resulting gritty paste to a pressure of 500 atmospheres in a hydraulic press, squeezed out a cell-free juice which possessed fermentative power. The enzyme responsible for fermentation was called zymase, and it has since been shown that it is present with many other enzymes (*e.g.*, invertase, emulsin, maltase, protease, peroxidase, catalase, dehydrogenase) in the powder called zymin, which is obtained by treating yeast cells with acetone and ether, and then drying the solid residue.

The first enzymic product separated from living cells always consists of a mixture of several enzymes and a host of accompanying inactive substances. The task of separating individual enzymes from one another, and freeing them from impurities, has raised difficult technical problems. The goal is the preparation of pure enzymes of known composition, and a very considerable concentration of various enzymes has been achieved.

Since the amount of enzyme present in a given product cannot yet be measured by ordinary quantitative methods of analysis, activity is taken as the criterion of concentration. Virtually by this is meant the amount of substrate that undergoes change in unit time when acted on, under defined conditions, by unit mass of the product possessing enzyme activity. For comparative experiments these conditions must be rigidly adhered to. Thus temperature *p*H (the optimum for the enzyme action under investigation should be used), and the initial concentration of substrate must be the same in every estimation. These and other factors, and the definitions of enzyme units of activity in present use, are considered by Haldane (*111*, p. 166). Starting with experiments on a given mass of living tissue, and proceeding to experiments on enzymic preparations of increasing purity obtained from this mass, investigators have found, as would be expected, that the *activity per unit mass* steadily increases, But the *total amount* of enzyme present may steadily decrease, owing to incomplete extraction from living cells and loss through various

causes during purification. Chief interest, however, at present resides not in yield but in activity, and the objective aimed at is to obtain a preparation which shows no further increase in activity per unit mass after subjecting it again to the purification process. Northrop succeeded in preparing from commercial pepsin a crystalline protein with a powerful peptic activity, which remained constant through seven successive crystallizations.

The data in table I make it clear that very considerable advances had been made before 1930 in the direction of preparing certain enzymes in pure form. The number given is the ratio of enzymic activity per gram dry weight of final preparation to that per gram dry weight of the original material.

TABLE I. *Concentration of enzymes*

Ricinus lipase . .	100	Jack Bean urease . .	730
Almond glucosidase . .	60	Horse-radish peroxidase.	20,000
Yeast saccharase . .	1,200	Aspergillus amylase .	30

It has for many years been the practice to concentrate certain enzymes (*e.g.*, malt diastase) by first adding alcohol to an aqueous extract of macerated tissue (*e.g.*, germinating barley) and then collecting the precipitate. This usually shows greater activity per unit mass than the original tissue. A simple illustrative exercise is to determine the times taken by equal masses of crude malt and purified diastase to cause starch to disappear completely from a given volume of a solution of given strength. Diastatic activity will be inversely proportional to the time.

Various methods (*e.g.*, fractional precipitation with alcohol, tannic acid, the salts of heavy metals, and solutions at different *p*H values; simple dialysis; electrodialysis; fractional sedimentation in a centrifuge; and differential inhibition or inactivation) have been employed in separating one enzyme from another, and in subsequent purification.

Willstätter and his co-workers developed adsorption methods and made notable advances, particularly in the direction of purifying plant peroxidase and invertase (see Haldane, *111*, and Waldschmidt-Leitz, *319*). These methods have since been much used in attempts to isolate enzymes (see Green, *100*, and Sumner and Somers, *loc. cit.*). Enzymes dispersed in aqueous solutions can, under suitable conditions, be adsorbed on the surfaces of certain substances (*e.g.*, varieties of aluminium hydroxide, kaolin, charcoal). It seems that adsorption is partly governed by electrical forces, for "the enzyme can generally, though not always, be eluted from the adsorbent by altering the *p*H and the charge on the enzyme, adsorbent, or both." Thus enzymes which are adsorbed in an acid reaction may be eluted with dilute

alkalies. Another method of elution is to displace the adsorbed enzyme by a substance for which the adsorbent shows higher chemical affinity. Thus phosphate acts as an eluent for alumina adsorbates, owing to the formation of aluminium phosphate.

The results of Willstätter's intensive study led him to conclude that enzymes are micellæ consisting of an active prosthetic substance associated with a colloidal bearer. In 1926 he asserted that enzymes do not belong to any of the known groups of complex organic compounds, but since that date knowledge of the chemistry of enzymes has increased rapidly. Sumner extracted defatted Jack Bean meal with 31·6 per cent. acetone, and filtered. He left the filtrate overnight in an ice chest, and obtained crystals of a protein which possessed intense urease activity. By recrystallization he prepared octahedral crystals which remained constant in composition and in urease activity when subjected to further processes of purification. Shortly afterwards Northrop obtained a crystalline protein possessing the activities of pepsin, and more recently (see Sumner and Somers, *loc. cit.*) crystalline proteins having in themselves enzyme properties have been prepared from crude amylase, plant proteases (*e.g.*, papain, ficin), trypsin, and other crude products. Crystalline proteins have also been obtained from other enzymes (see below), which require a prosthetic group before they show their activity. These achievements represent a big advance in the direction of purification; but Stern maintains that crystalline form and constancy of activity upon recrystallization must not be taken in the protein group as a sufficient criterion of the presence of a single substance; crystalline enzymes or components of enzymes may possibly consist of two or more proteins, and "it cannot be excluded that more refined methods might still reveal a prosthetic group in these enzymes." They would then accord with Willstätter's picture of an enzyme, as do those we shall now describe.

The activity of certain enzymes depends upon the presence of a prosthetic group as well as of a protein component or components. The whole enzyme is a conjugate protein, and is sometimes described as a proteid. Thus iron-porphyrin compounds (hæmochromogens) are the prosthetic groups of cytochrome oxidase, catalase, and peroxidase; copper in organic combination has been found in the polyphenol oxidase obtained from potato tubers; alloxazine nucleotide (riboflavin-phosphoric acid) is the active group in the yellow enzyme; some dehydrases require for their action the diphospho- or triphospho-pyridine nucleotides known as codehydrase I (cozymase) and codehydrase II; and the presence of pyrimidine-thioazole-phosphoric ester (cocarboxylase) is necessary for carboxylase activity. Some of these substances have been prepared in the pure state, and their constitutions established by analysis and synthesis. Highly

important advances in knowledge have resulted from the use of such purified active substances in biochemical experiments.

It has been suggested that a prosthetic group is attached to a protein through the heavy metal in the hæmochromogen and copper compounds, and through the phosphoric acid residues in the others. Theorell demonstrated the reversible dissociation of the yellow enzyme into protein and alloxazine-nucleotide, and he resynthesized the enzyme from its components. Warburg carried out similar experiments with dehydrases containing pyridine nucleotides. These successes represent noteworthy advances, but we cannot speak of the true synthesis of such enzymes until chemists succeed in synthesizing the specific proteins of the enzymes concerned from their elements. The present position is that in these reconstructed conjugate proteins the protein component is exclusively a metabolic product, but the prosthetic substance has in certain instances been a synthetic product.

It should be noted that some of the prosthetic groups of these active conjugate proteins have been described as coenzymes. A *coenzyme* has been defined as a heat-stable crystalloidal organic component of a complex enzyme system, which is necessary for the display of at least one catalytic activity of that system. Cozymase (codehydrase I) was the first coenzyme to be discovered; the properties of cophosphorylase (adenosine triphosphoric acid), cocarboxylase, and codehydrase II, have been described in recent years. Euler denotes a complete complex enzyme by the term *holoenzyme*, and the parts by the terms *apoenzyme* and *coenzyme*. In addition inorganic components may be necessary for full activity, *e.g.*, phosphates and magnesium salts are necessary for full zymase activity.

It is evident that for full activity the structure of both the apoenzyme and coenzyme components must be in working order. Interference with the structure of either would result in partial or complete inhibition of the holoenzyme. In the development of knowledge of complex enzyme actions differential inhibition of components has played an important part. For example, the action of prosthetic groups containing heavy metals can be stopped by hydrogen cyanide and by hydrogen sulphide; evidently, therefore, the complete action of holoenzymes containing as prosthetic groups iron-porphyrin or copper in organic combination will not be shown in the presence of these poisons.

C. Notes on Hydrolytic Enzymes

Classification. In this group are included those enzymes (*e.g.*, esterases, carbohydrases, proteolytic enzymes, and amidases) which catalyze hydrolyses.

I. ESTERASES. The hydrolysis of substances containing an ester linkage with the formation of free acids and alcohols are catalyzed by esterases.

Lipase is found in resting and germinating seeds containing fatty oil as reserve food. It is usually stated, however, that plant lipase is insoluble in water. It has been suggested that either the enzyme itself is proteinaceous and insoluble, or is adsorbed on an insoluble proteinaceous bearer. *In vitro* it hydrolyzes fats, giving free fatty acids and glycerol. The properties of lipase in resting castor oil seeds are slightly different from those of lipase in germinating seeds. The latter is active in neutral and even in a weakly alkaline solution, while the former displays activity only in acid solution, the optimum *p*H being 4·7 to 5·0. Some authorities regard the lipase in resting seeds as a pro-enzyme or zymogen, which is activated by hydrogen ions. Among the *phosphatases* are placed the enzymes which, by hydrolysis, dephosphorylate esters (*e.g.*, hexose-phosphates, cocarboxylase, lecithin, phytin) with the liberation of phosphoric acid. No phosphate acceptor is required (*cf.* p. 53). *Chloro-phyllase* is present in green leaves, and cleaves chlorophyll. In the presence of ethyl alcohol, ethyl chlorophyllid, which is crystallizable, is produced.

II. CARBOHYDRASES. In this group it is convenient to separate the polyases, which hydrolyze polysaccharides, from the glycosidases, which cause hydrolytic cleavage of glycosidic linkages in di- and tri-saccharides. It must be noted, however, that polyases may act on glycosidic linkages in the giant polysaccharide molecules.

(i.) POLYASES. *Amylases* or *diastases* are probably present in all living cells that contain starch. Their occurrence in germinating barley or in amyliferous leaves (*e.g.*, those of leguminous plants) is easily demonstrated. In certain leaves, however, the presence of tannins may depress activity. Malt amylase is obtained from germinating barley. *In vitro* it catalyzes the hydrolysis of starch with the production of erythrodextrin, achroodextrin, and maltose. It is easy by experiment to demonstrate the successive disappearance of starch and erythrodextrin, substances which respectively give blue and red colours with iodine ; and, if a sugar-free diastase is used, to detect the production of the reducing sugar maltose, which yields a characteristic osazone. In such experiments about twenty-five per cent. of the products of hydrolysis consists of achroodextrin, which is insoluble in alcohol, and consequently can be readily separated from maltose. Evidence exists (see Freeman and Hopkins, *93*, and Hanes, *114*) that there are three amylases (*a*) a liquefying amylase, which disaggregates starch particles to produce macromolecules of starch (p. 433) ; (*b*) a β-amylase, which splits maltose directly off from the starch macro-molecules ; and (*c*) an α-amylase, which causes dextrins to accumulate before maltose appears. Malt contains both α- and β-amylase, but ungerminated barley contains only β-amylase. In the presence of a thermostable substance of unknown composition, which is known as *complement*, amylases show extended activity. They then readily hydrolyze dextrin. Possibly the fact that dextrins are never found in the free state *in vivo* may be accounted for by the association of amylase with complement in living cells. Autolyzing yeast serves as a source of complement.

Cellulase occurs in many fungi and bacteria, and may be present in the germinating seeds of certain flowering plants. It hydrolyzes cellulose to

form glucose, cellobiose being an intermediate product. *Lichenase* occurs in lichens and some germinating seeds (*e.g.*, barley). It cleaves lichenin to form cellobiose.

Hemicellulases or *cytases* are found in seeds (*e.g.*, date) that contain hemicelluloses as reserve food. It is probable that this group will be resolved into several individual enzymes. Thus since cytases can cleave galactans to form galactose, and mannans to form mannose, they may contain specific galactanases and mannanases. *Inulase* has been detected in the sprouting buds of storage organs (*e.g.*, the tubers of the Jerusalem artichoke) containing inulin as a reserve food. It effects the hydrolysis of inulin to fructose.

(ii.) GLYCOSIDASES. *Maltase* always occurs with amylase in the cells of higher plants, and, if they act in conjunction, maltase must be a vigorous enzyme, seeing that maltose has not often been found in the free state in plants. Maltase hydrolyzes this disaccharide to glucose. It may also hydrolyze other α-glucosides (*e.g.*, α-methyl-glucoside). *Emulsin* occurs in almonds, and hydrolyzes many β-glucosides (*e.g.*, amygdalin, salicin). It is compounded of at least two enzymes, namely, *amygdalase*, which cleaves amygdalin to form glucose and prunasin, and *prunase*, which cleaves prunasin to form glucose, benzaldehyde, and hydrogen cyanide (which is volatile and may be detected by the browning of moist sodium picrate paper). It is probable that the specificity of emulsin towards β-glucosides must be attributed to the properties of the prunase component.

Invertase (*sucrase, saccharase*) is present in yeast, and in the shoot- and root-systems of higher plants. It may readily be shown that it hydrolyzes cane sugar (a non-reducing sugar which does not form osazones) to reducing sugars from which glucosazone can be prepared. The hydrolysis may be followed with a polarimeter. Cane sugar is dextrorotatory, and the mixture of the products of hydrolysis, viz., equal amounts of glucose and fructose, lævorotatory, since glucose is less dextrorotatory than fructose is lævorotatory. Because of the change of rotation this mixture was termed invert sugar, the hydrolysis was described as an inversion and the enzyme received the name invertase. Invertase can also act on trisaccharides containing the same linkage between glucose and fructose as occurs in cane sugar. For example, raffinose is hydrolyzed to fructose and melibiose. *Melibiase* has been detected in almonds. It hydrolyzes melibiose into glucose and galactose, and cleaves the linkage between glucose and galactose in raffinose, so yielding cane sugar and galactose as products of hydrolysis. *Cellobiase* is associated with lichenase in the cells of green plants. It hydrolyzes cellobiose to glucose.

III. PROTEOLYTIC ENZYMES. All enzymes which take part in the hydrolysis of proteins to peptones, polypeptides, and amino acids, belong to this group, and possess in common the power of effecting the hydrolytic cleavage of the polypeptide linkage with the formation of free amino and carboxylic groups. Until recently they were divided into two classes, viz., the *pepsins* and the *erepsins*. It was supposed that the pepsins hydrolyze proteins to peptones, and the erepsins peptones to amino acids. At present a division into proteases and peptidases appears to be favoured.

Proteases are easily detected in resting and germinating seeds, green leaves, etc., by their power of dissolving proteins (*e.g.*, blood fibrin). Not only do they dissolve but they hydrolyze proteins with the formation of

polypeptides. Certain proteases, for example *papain* of the melon tree (*Carica papaya*), and *bromelin* of the pineapple, have for long been separated from other enzymes of this class because of their peculiar properties. Thus recent researches have shown that papain in the presence of hydrogen cyanide, which acts as a coenzyme, can hydrolyze polypeptides, *i.e.*, whereas proteins can undergo hydrolysis only as far as polypeptides in the presence of papain, amino acids are produced when papain-hydrocyanic acid is employed.

Peptidases are associated with proteases in resting and germinating seeds, green leaves, etc. They cannot cleave proteins, but act on the products formed by the action of proteases on proteins. It was at one time thought that a single enzyme, erepsin, brings about the second phase in protein degradation, viz., that by which amino acids are produced. Specific peptidases have been found in animal cells and yeast, and it is probable that similar enzymes are present in the higher plants. In yeast a *polypeptidase* cleaves polypeptides to form dipeptides, and a *dipeptidase* effects a further hydrolysis, which liberates amino acids. The presence of erepsin (*i.e.*, a mixture of peptidases) can be detected by allowing the enzyme preparation to act on a peptone (*e.g.*, Witte's peptone), and demonstrating the production of amino acids. Among these tryptophane is readily recognized, since it is acted on by bromine water to give a pink product, which is soluble in amyl alcohol.

IV. AMIDASES. Amidases govern the hydrolytic deamidation of amides. Ammonia and an acid are produced. The reaction may be reversible.

$$R.CO.NH_2 + H_2O \rightleftarrows R.COOH + NH_3$$

Urease, the first enzyme to be prepared as a crystalline protein (p. 23), has been found in many higher plants ; it hydrolyzes urea to form carbon dioxide and ammonia. When attacked by *asparaginase*, which Chibnall and Grover separated from germinating barley, asparagine yields aspartic acid and ammonia. *Glutaminase*, demonstrated by Vickery *et al.* as present in the roots of beet, controls the hydrolytic deamidation of glutamine to produce glutamic acid and ammonia. Evidence exists that asparaginase and glutaminase can act synthetically (p. 69), and it may be confidently affirmed that these enzymes are present in all cells which produce or consume asparagine and glutamine.

Syntheses by hydrolytic enzymes. It follows from the law of mass action (p. 421) that the equilibrium position attained in a reversible reaction is governed by the initial concentration of the reactants. Hence in a reversible hydrolysis $\rightleftarrows$ condensation reaction, hydrolysis would tend to be favoured in dilute solution, and condensation in strong solution. Now enzymes as catalysts are supposed to accelerate opposed reversible reactions to the same extent, and thus not to affect the final equilibrated state. Consequently it appeared to van't Hoff (1898) that hydrolytic enzymes might under suitable conditions catalyze reactions in the direction of synthesis. In the same year Croft-Hill, then a medical student, established this contention by experiment, when he synthesized maltose from a strong solution of glucose under the agency of yeast maltase. Later

investigations have shown that Croft-Hill's condensate was a mixture of maltose and isomaltose, and that the production of the latter form, a β-glucoside, was attributable to the presence of emulsin in the enzyme preparation used. Owing principally to the work of Bourquelot and Bridel, it is now clear that emulsin (or its component prunase) is capable of synthesizing the same β-glucoside as it hydrolyzes, and that maltase can synthesize other α-glucosides besides maltose. Thus it appears that the specificity of hydrolytic enzymes extends to the products of hydrolyses as well as to condensates.

Other syntheses which have been established beyond doubt and much investigated (see Bayliss, *19*) are those of esters (*e.g.*, amyl butyrate) and fats (*e.g.*, tri-olein) by lipase. At various times it has been asserted that syntheses have been effected by invertase, proteases, and other hydrolytic enzymes, but the evidence is not yet generally accepted as satisfactory. Experimental investigations present great difficulties, because in aqueous solutions *in vitro* hydrolysis rather than synthesis is favoured. But as Bayliss (*loc. cit.*) has pointed out, even a small amount of synthesis may be of great importance, since, in a living cell, the condensate may be removed by further metabolism, diffusion, precipitation, etc., as soon as it is produced. Under such circumstances condensation rather than hydrolysis would be favoured. Moreover, it may be that *in vivo* synthesis by enzymes is promoted in certain phases of protoplasm (*e.g.*, lipoid phases) owing to the fact that only a small amount of chemically active water is present.

D. Enzymes and Carriers concerned with Oxidations and Reductions [1]

Many substances which are stable in the presence of molecular oxygen rapidly undergo aerobic oxidation in living cells. It has consequently been inferred that every living cell contains enzymes which occasion oxidation by activating either oxidizable substances, or molecular oxygen or some other oxidizing agent. Excepting when oxidation occurs by the addition of molecular oxygen—apparently a rare event in living cells—the oxidizing substance, in effecting oxidations, becomes reduced (see p. 418). Thus the same enzyme system may be concerned with oxidation and reduction. For example, there is present in the cells of potato tissue an enzyme which oxidizes certain aldehydes (*e.g.*, acetaldehyde) in the presence of nitrates, with the reduction of the latter to nitrites.

Direct and indirect oxidases. It can readily be demonstrated that protoplasm possesses powers of oxidation. About fifty per cent. of the higher plants can effect the aerobic oxidation of gum guaiacum

[1] The books written by Green (*100*) and by Sumner and Somers (*loc. cit.*) are recommended for further reading about this fundamental subject.

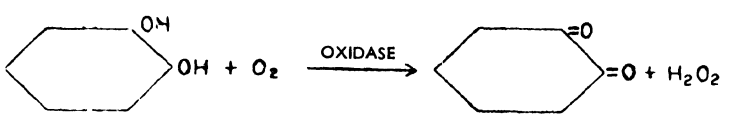
(a substance which is not autoxidizable) dissolved in alcohol, and a blue product is formed. There can be separated from such plants (*e.g.*, potato) an enzyme system which effects the direct blueing of guaiacum in the presence of oxygen. It is called the *direct oxidase* system. Cells possessing this type of system become discoloured after injury. Thus brown colours may develop as a result of the enzymic oxidation of polyphenolic substances dissolved in cell sap (*e.g.*, in autolyzing cherry laurel leaves); and other tissues (*e.g.*, potato, broad bean) may turn first red and then black, owing to the action of a peculiar oxidase *tyrosinase* which oxidizes tyrosine.

It appears that these direct oxidases, of which there are many varieties, are complex systems. They have been the subject of extensive work, and some controversy (see Onslow, *214*, chap. III, Boswell and Whiting *44*, and Graubard, *98*). Resulting from the work first of Onslow and later of Szent-Györgyi it is now established that a system termed *catechol oxidase* is responsible for the direct blueing of guaiacum, and the oxidation of polyphenols. A comparable system, but presumably of different composition, is present in yeast, bacteria, and the cells of higher animals. It oxidizes the so-called nadi reagent (a mixture of α-naphthol and dimethyl-paraphenylenediamine) to indophenol blue. It used to be called the *indophenol oxidase.* It is now known that the system only shows its activity in the presence of cytochrome, which actually oxidizes the nadi reagent. Accordingly the enzymic part of it has been named *cytochrome oxidase.* This enzyme system is probably identical with Warburg's respiratory enzyme (see below), and owes its oxidative activity to its prosthetic group, which is an iron-porphyrin compound. Doubt still exists concerning the chemistry of the prosthetic group of catechol oxidase. The name *polyphenol oxidase* (see Green, *100*) has recently been given to an enzyme, occurring in potato tubers, which has oxygen and polyphenols for its specific reaction partners. Kubowitz has purified this enzyme, and shown that it is a copper proteid. The most active preparation contained 0·165 per cent. copper, and the activities of Kubowitz's preparations were governed in linear fashion by their copper content, his results indicating that in the absence of copper the preparations would be inactive. The copper proteid can oxidize catechol, but it is not yet established that all catechol oxidases are copper proteids. It would be interesting to know whether plants that normally contain catechol oxidase can be grown in culture solutions completely free from copper (*cf.* p. 169), and, if growth occurred, whether the developing plants contained catechol oxidase. Clearly, were this enzyme present it could not be a copper proteid; were it absent the inference would be that catechol oxidase did not play an essential part in the metabolism of the experimental plants.

Cells of such plants (*e.g.*, horse-radish) as cannot directly occasion the aerobic oxidation of gum guaiacum, in common with all living cells, contain an enzyme *peroxidase* which activates hydrogen peroxide, and thereby greatly enhances the oxidizing powers of this substance. For example, neither horse-radish root alone nor hydrogen peroxide alone can oxidize guaiacum, but a blue colour develops if both guaiacum and hydrogen peroxide are applied to the cut surface of horse-radish root. Coloured products are also given if phenolic substances (*e.g.*, pyrogallol, catechol, quinol), benzidine, or *p*-phenylene diamine are used instead of guaiacum. Pyrogallol is oxidized to purpurogallin. The amount of this substance produced in unit time under specified conditions gives what is called the purpurogallin number, which is a measure of the peroxidase activity. For details see Green (*loc. cit.*). All peroxidases contain iron-porphyrin (*i.e.*, hæmatin) prosthetic groups. A thermostable catalyst showing peroxidase activity may occur in plant cells. It also is a hæmatin.

Plants containing peroxidase, but not direct oxidase, have been classed as *indirect oxidase* plants, because they will not blue guaiacum until hydrogen peroxide has been added. Such plants, when injured, do not turn brown. It should be noted that on account of the presence of peroxidase, colour changes brought about by direct oxidase plants may be intensified upon adding hydrogen peroxide.

It appears that a direct oxidase, considered as a system which causes the colour changes we have described, is compounded of two enzymes, viz., catechol oxidase and peroxidase, and that the essential properties of a direct oxidase may be attributed to the *catechol oxidase* component. This enzyme is a *dehydrase* or *dehydrogenase*, and activates a cellular substance containing a catechol grouping, which then becomes converted by dehydrogenation into an orthoquinone. It is supposed that molecular oxygen is also activated and then functions as a hydrogen acceptor, *i.e.*, it is reduced. For catechol itself one may summarize the reaction thus :



Orthoquinones are very strong oxidizing agents, and can themselves effect all the colour changes by which direct oxidases are characterized. It is also possible, however, that peroxidase will immediately act on the hydrogen peroxide, and thus contribute to the oxidation powers of direct oxidases. According to this view a system compounded of a catechol compound, catechol oxidase, molecular oxygen, and peroxidase, could be responsible for the colour changes.

The cellular functions of the direct and indirect oxidases have not yet been elucidated. One point requires stressing : whereas catechol compounds may accumulate in relatively high concentrations in cells containing catechol oxidase, orthoquinones do not. Either the orthoquinones are not produced, or, if they are, they are immediately reduced or consumed in some other way. As carriers in a hydrogen transfer system immediate reduction would be their fate. Clearly, a fundamental question is to determine which of the naturally occurring substances can donate hydrogen to orthoquinones, and so themselves become oxidized by dehydrogenation. Some such reducers (*e.g.*, ascorbic acid, p. 41) are known, but it has not been established that any of them are effective *in vivo*.

Catalase. Hydrogen peroxide is produced when molecular oxygen acts as the hydrogen acceptor in oxidations by dehydrogenation, as, for example, in the action of catechol oxidase on catechol. The enzyme catalase is invariably present in the cells of aerobic organisms, but is absent from those of strictly anaerobic bacteria. Inasmuch as it decomposes the peroxide as soon as it is produced, catalase has the functional value of preventing this compound from exerting a toxic effect. It should be noted that molecular oxygen is liberated (*cf.* peroxidase action).

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2.$$

Catalase is a proteid consisting of a protein, which has been obtained in crystalline form, combined with a hæmochromogen of known composition. Its activity, like that of other enzymes containing heavy metals, is inhibited by hydrogen cyanide and by hydrogen sulphide.

Warburg's respiratory enzyme. Many years ago Warburg strongly urged that the activation of molecular oxygen is a necessary preliminary to such oxidations as are accompanied by oxygen uptake. He attributed this activation to the properties of a single respiratory enzyme, and portrayed this enzyme as a sort of organic colloidal micella containing iron. He held that the function of the iron is to activate the oxygen (see (i.) below), and that the oxidations subsequently occur on the surface of the micella (see (ii.) below).

$$\text{X.Fe (respiratory enzyme)} + O_2 = \text{X.Fe.O}_2 \quad . \quad . \quad . \quad . \quad . \quad \text{(i.)}$$
$$\text{2A (respirable substrate)} + \text{X.Fe.O}_2 = \text{2AO} + \text{X.Fe.} \quad . \quad . \quad . \quad \text{(ii.)}$$

He obtained evidence that such a catalytically active complex containing iron is present in the respiratory system of living cells. Thus he found that the extent to which cell respiration is inhibited by narcotics (*e.g.*, phenyl urethane) is proportional to the amount of narcotic which is adsorbed on the intracellular surface, and concluded that respiration is a surface reaction.

Now hydrogen cyanide and hydrogen sulphide inhibit (*a*) chemical reactions that are catalyzed by heavy metals (including iron), and (*b*) cell respiration as judged by oxygen uptake. Warburg's quantitative experiments showed that these poisons had inhibitory effects which were far greater than could be accounted for by surface inactivation. Thus hydrogen cyanide in M/10,000 solution markedly depressed respiration. Warburg concluded that such depression occurs because cyanides and sulphides combine with the iron in the respiratory enzyme, and thus prevent the metal from taking part in the activation of oxygen.

From the results of his investigations on the properties of different charcoals, Warburg adduced further evidence in support of his view that respiration is a surface reaction in which iron acts as a catalyst. He found that charcoals (*e.g.*, blood charcoal) containing iron and an organic nitrogen compound could, in aqueous solutions, catalyze the oxidation by molecular oxygen of amino acids and oxalic acid. He also showed that catalysis by these cell models was inhibited by narcotics proportionately to, and by cyanides out of all proportion to, the surface occupied.

The fact that carbon monoxide inhibits cell respiration suggested to Warburg that the respiratory enzyme is a hæmatin derivative, since these derivatives form additive compounds with carbon monoxide. It appears that these additive compounds of hæmatin are dissociated by light. Hence great significance attaches to Warburg's discovery that the respiration of yeast cells, which had previously been depressed by treatment with carbon monoxide, was restored to full activity by illuminating the cells. By showing that only those wavelengths of light that correspond to the absorption bands in the CO-hæmochromogen spectrum can thus restore respiration, Warburg established beyond reasonable doubt that hæmatin derivatives play a part in the respiratory mechanism.

It is widely accepted that there are present in living cells oxidizing enzymes of the kind that Warburg has so thoroughly investigated. But it is not generally agreed that active systems containing hæmatin or copper in organic combination provide the only enzymes that play a part in respiratory oxidations.

Dehydrases. The work of Thunberg and others has in more recent years provided much evidence that Wieland's views on oxidation by dehydrogenation are applicable to respiratory events in living cells. It is supposed (*a*) that labile hydrogen in a respirable substrate (AH_2) is acted on by an enzyme that is alternatively described as a dehydrogenase or a dehydrase, and (*b*) that this activated hydrogen may be transported to any hydrogen acceptor (B) that has a higher affinity for hydrogen than the affinity possessed by A. As a result of this transfer of the activated hydrogen, the hydrogen donator, AH_2, is

oxidized by dehydrogenation to a substance **A**, and the hydrogen acceptor B is reduced to BH_2.

$$(AH_2 + \text{dehydrase}) + B \rightarrow A + BH_2$$

In such oxidoreductions we shall often refer to the system (AH_2 + dehydrase) as the dehydrase system.[1] It is important to realize that neither the substrate AH_2 alone nor the dehydrase enzyme alone can reduce B. Reduction of B requires the presence of both an oxidizable substrate and the proper enzyme. Plainly it follows that for the oxidation of a substrate a suitable hydrogen acceptor must be present as well as the proper dehydrase enzyme. The above reaction would continue until B is all reduced to BH_2, and would then stop. When hydrogen acceptors are present in very low concentrations, as happens usually, the regeneration of B from BH_2 is a necessary condition for the complete oxidation of AH_2. The cytochrome system will provide us with an example of how this may be brought about, with oxygen, which is in unlimited supply, as the ultimate hydrogen acceptor.

The activity of certain simple dehydrases, and of more complex systems (*e.g.*, the cytochrome system) containing dehydrases, may be determined by measuring the rate of oxygen uptake in a Barcroft-Warburg manometer (p. 263). The activity of formic dehydrase (p. 40) provides an example. Alternatively Thunberg's beautifully simple method may be used. This depends upon the fact that under anaerobic conditions methylene blue may accept hydrogen from certain donators and become reduced to leuco methylene blue. Thunberg carried out the reductions in specially designed tubes from which air could be readily evacuated, and determined the activity of the dehydrase from the time taken for the blue colour to disappear. Methylene blue is rapidly decolorized by the donators and dehydrases that act together in yeast, and in many animal tissues. The donators may be removed from yeast, animal tissues or from certain plant tissues by washing with water. With the removal of such oxidizable subtrates the rate of decolorization decreases. Then the types of dehydrase enzymes present may be determined by the addition to the washed cells or tissues of substances that are selected because they are potential hydrogen donators. For instance, Thunberg (see Onslow, *214*, p. 152) found that washed seeds of mallow, orange, and plum, decolorize methylene blue in the presence of oxalates, and inferred that the seeds contained an oxalic dehydrase. Further, he reported the presence of malic, formic, and succinic dehydrases in the seeds of the runner bean, and of citric dehydrase in the seeds of cucumber. For several reasons dehydrases are not so easily detected

[1] The two names *dehydrase* and *dehydrogenase* appear to be used indiscriminately. The latter describes the enzyme more precisely. Preference is often shown for the shorter word, when the enzyme has been resolved into apodehydrase and codehydrase.

in the cells of higher plants as they are in animal cells, yeast and bacteria, but evidence concerning them is increasing. For example, there is a glutamic acid dehydrogenase in leguminous seedlings (see p. 48), and a lactic dehydrogenase has been detected in germinating barley (see p. 43).

It is known that purely chemical oxidations by dehydrogenation may occur both in the presence and absence of oxygen. Thus Wieland found that glucose in aqueous solutions containing palladium as a catalyst is, at room temperature, oxidized by the molecular oxygen of the air. He also found that the oxidation proceeded anaerobically in the presence of quinone ($C_6H_4O_2$). This substance acted as a hydrogen acceptor (*i.e.*, as an oxidizing agent) and during the course of the reaction was reduced to hydroquinone ($C_6H_6O_2$). Wieland concluded that atmospheric oxygen acted as a hydrogen acceptor in the aerobic oxidation. A point to notice again is that hydrogen peroxide is the initial product when molecular oxygen acts as the acceptor of activated hydrogen.

Dehydrase systems in living cells belong to two classes. In the first class are included the systems that can co-operate directly with the molecular oxygen of the air. These are called *aerobic dehydrase systems*. Tissues containing such systems absorb oxygen. This element then acts as the hydrogen acceptor of the labile hydrogen of the dehydrase system. The reaction may proceed thus :—

$$(AH_2 + \text{dehydrase}) + O_2 \rightarrow A + H_2O_2$$

The fate of the hydrogen peroxide may be twofold. First, it may be acted upon by peroxidase, which is universally present in living cells, and yield atomic oxygen. This intensely active form of oxygen may then effect various cell oxidations. Some experimental support for this idea of the coupling of aerobic dehydrase systems with peroxidase has already been obtained. Secondly, hydrogen peroxide may be acted on by catalase (see above).

A point of great importance is that aerobic dehydrases do not, as a rule, show the sensitivity to cyanides that Warburg believes to be a property of his respiratory enzyme. This indicates that aerobic dehydrases are distinct from Warburg's respiratory enzyme, *i.e.*, dehydrases are not micellæ containing iron or copper. Dixon and Elliot showed that (*a*) the oxygen uptake of yeast was not completely inhibited by M/10 hydrogen cyanide, and (*b*) the maximum inhibition for animal cells was, on the average, about 60 per cent. They attributed the residual oxygen uptake to the activity of aerobic dehydrase systems. It appears therefore that, besides Warburg's respiratory enzyme, there are other systems which play a part in respiratory oxidations.

The term *anaerobic dehydrase system* is used to describe the second

class of dehydrase system. Such a system cannot co-operate directly with the molecular oxygen of the air, *i.e.*, molecular oxygen cannot act as the hydrogen acceptor of the labile hydrogen in the system AH_2 plus anaerobic dehydrase. There must be present some substances or systems with a higher affinity for hydrogen than that possessed by molecular oxygen. The investigation of such substances or systems has been a major biochemical research.

The presence of anaerobic dehydrases is readily detected by means of methylene blue; for example, the oxidation of lactic acid to pyruvic acid in yeast, certain bacteria, and animal tissues, takes place anaerobically in the presence of methylene blue :—

$$(\text{Anaerobic dehydrase} + CH_3 \cdot CHOH \cdot COOH) + MB \rightarrow CH_3.CO.COOH + MBH_2$$

As the lactic acid is not directly oxidized if molecular oxygen is substituted for methylene blue, it is concluded that the dehydrogenation is governed by an anaerobic dehydrase. A point to notice is that none of the known dehydrases contains catalytically active iron centres, and, consequently, none is inactivated by hydrogen cyanide. As we shall see below (p. 37), in the anaerobic dehydrase systems, it is the other enzymic component, essential for oxygen uptake, which is sensitive to cyanide.

Respiratory oxidations in aerobic organisms are always accompanied by oxygen uptake. The question arises whether such oxidations are in any way related to the activity of anaerobic dehydrase systems. Before this question can be profitably discussed in chap. XIV, we must consider the properties of certain cell substances which may be connecting links between these dehydrase systems and systems under the control of Warburg's respiratory enzyme.

Keilin's cytochrome system. Keilin discovered by spectroscopic methods that three closely allied hæmochromogens, which he named cytochromes *a*, *b*, and *c*, were present in the living cells of all the aerobic organisms he examined. It appears that, in the presence of oxygen, these forms of cytochrome exist in an oxidized state. When, however, the supply of oxygen is cut off, they are rapidly reduced. It is a simple matter to recognize the process of oxidation and reduction, as the absorption spectrum of the reduced forms contains four distinct bands which are not present in that of the oxidized forms. To examine the spectrum of reduced cytochrome, place a flat-sided vessel containing a yeast suspension in an aqueous solution of sodium hydrosulphite, between a source of bright light (*e.g.*, light of the arc lamp) and the spectroscope. The yeast suspension should have the consistency of a paste.

Keilin has investigated certain well-known oxidations, brought

about by animal cells and yeast, in which anaerobic dehydrases are believed to play a part, and there is a continuous uptake of oxygen. The results obtained indicate that cytochromes *a* and *c* act as intermediate carriers of hydrogen. Keilin's view is that oxidized cytochrome acts as a hydrogen acceptor of the labile hydrogen in an anaerobic dehydrase system. As a result all the cytochrome tends to pass into the reduced form.

$$(AH_2 + \text{anaerobic dehydrase}) + Cyt \rightarrow A + CytH_2$$

Thus in the presence of phenylurethane, oxidized cytochrome cannot be reduced owing to the fact that dehydrases are inhibited by certain narcotics.

Were there no mechanism in the cell for re-oxidizing reduced cytochrome, this intermediate carrier would remain in the reduced state. We have already seen that this happens under anaerobic conditions, and we shall point out below that this also happens under aerobic conditions in the presence of cyanides. Under natural conditions, however, cytochrome exists in aerobic organisms partially, at least, in the oxidized form. Now it appears that, whereas cytochrome *b* is oxidized by molecular oxygen, cytochromes *a* and *c* are not autoxidizable substances; hence we cannot attribute the re-oxidation of such cytochrome as has just been reduced during the dehydrogenation of a metabolite to the direct action of the molecular oxygen that has been absorbed from the environment. It is at this stage, according to Keilin, that the system governed by Warburg's respiratory enzyme plays its part in respiratory metabolism. He believes that the function of this enzyme is not, as Warburg has stated, to co-operate with oxygen in bringing about the oxidation of respirable substrates, but to co-operate with oxygen in the oxidation of reduced cytochrome:

$$CytH_2 + (\text{Warburg's respiratory enzyme} + O_2) \rightarrow Cyt + H_2O_2$$

The regenerated oxidized cytochrome can then once more act as the hydrogen acceptor in an anaerobic dehydrase system. Plainly the contention is that respirable substrates are oxidized by dehydrogenation when the complete complex cytochrome system is active, the hæmochromogens functioning in the transfer of hydrogen.

Keilin did not use the term respiratory enzyme for the system that governs the oxidation of reduced cytochrome. He referred this oxidation to the activity of indophenol oxidase in animal cells and yeast, and to catechol oxidase in the higher plants. The fact that indophenol oxidase and catechol oxidase are inhibited by cyanides and sulphides accorded with this view, seeing that the cells treated with cyanides or sulphides cannot under any conditions oxidize reduced cytochrome. Still stronger evidence was obtained when Keilin succeeded in oxidizing reduced cytochrome *c* by means of the

indophenol oxidase of yeast. The name indophenol oxidase has now been replaced by the name cytochrome oxidase (see p. 29).

$$(CytH_2 + \text{cytochrome oxidase} + O_2) \rightarrow Cyt + H_2O_2$$

The aerobic oxidation of succinic acid to fumaric acid provides an example of an oxidation by the complete complex of the proper dehydrase, cytochrome, and cytochrome oxidase. The name succinoxidase was at one time given to this particular complex. Experiments showed that whereas the action of this oxidase was inhibited by cyanides when molecular oxygen was used as hydrogen acceptor, succinic acid could be oxidized in the presence of cyanides when methylene blue was used as the acceptor. This difference can now be readily explained. Methylene blue virtually replaces the complex system [cytochrome, cytochrome oxidase, and molecular oxygen]; hence the oxidation is brought about by the dehydrase system, which is insensitive to cyanides, coupled with the methylene blue. The aerobic oxidation, however, depends upon the activation of the oxygen which is absorbed, and this activation requires the presence of active centres of iron in the cytochrome oxidase. Cyanides inactivate these centres, and as a result the aerobic oxidation of succinic acid is inhibited.

This continuous oxidation would, according to Keilin, proceed in the following stages :—

(*a*) Activation of hydrogen in succinic acid by anaerobic dehydrase. This continues as long as the succinic acid remains in the reacting system.

(*b*) Transfer of the activated hydrogen to oxidized cytochrome, which is thereby reduced. The succinic acid would be oxidized by dehydrogenation to fumaric acid.

$$(\text{Anaerobic dehydrase} + COOH.CH_2.CH_2.COOH) + Cyt \rightarrow COOH.CH:CH.COOH + CytH_2$$

This reaction would continue as long as some oxidized cytochrome remained in the reacting mixture.

(*c*) The oxidation of reduced cytochrome by oxygen acting with cytochrome oxidase. The regenerated oxidized cytochrome then acts again as in (*b*) on the activated succinic acid produced as in (*a*). It is possible that during the oxidation of reduced cytochrome, hydrogen peroxide may be produced. This compound, however, will not accumulate, as catalase is also present in the tissue. Peroxidase may also act on the hydrogen peroxide to yield active oxygen (*cf.* p. 30).

The oxidation we have just considered, which was first studied in animal tissues, has acquired a new interest for botanists since Hill and Bhagvat (*130*) reported they had detected cytochrome and succinic dehydrase in cotyledons of germinating Phaseolus seeds.

Holodehydrase systems composed of apodehydrases and codehydrases. In 1934 Warburg and Christian announced the discovery in red blood-corpuscles of a coenzyme containing the pyridine compound, nicotinic acid amide (p. 440). The wide distribution in plant and animal cells of two complex chemicals containing this amide and possessing coenzyme properties has since been reported. These two coenzymes have been

separated in crystalline form, and the constitution of each compound has been determined by analysis and synthesis. One of them, viz., codehydrase I, is identical with Harden and Young's cozymase (p. 50). It is a dinucleotide (p. 444), but as a chemical substance is still referred to as diphosphopyridine nucleotide. The other, codehydrase II (Warburg's coenzyme), possesses an extra phosphoric acid residue. It, also, is a dinucleotide, but is described as triphosphopyridine nucleotide.

These coenzymes are the prosthetic groups of certain dehydrases, each complete enzyme (holodehydrase) being composed of a proteinaceous apodehydrase combined with cozymase or with Warburg's coenzyme, through one of the phosphoric acid residues in these nucleotides. The apodehydrase probably activates two hydrogen atoms in its proper substrate, and the codehydrase accepts these atoms, the diphospho or triphosphopyridine nucleotide being reduced to diphospho or triphospho-dihydropyridine nucleotide.

$$AH_2 + \text{codehydrase} \xrightarrow{\text{apodehydrase}} A + \text{dihydrocodehydrase}$$

The specificity of the holodehydrase is determined by the properties of the proteinaceous apodehydrase component; the same codehydrase may be active in different dehydrase systems.

Important pioneering studies were made on the activity of codehydrase II, which, in association with one specific apodehydrase, oxidizes Robison's ester (hexose monophosphate), and with another specific apodehydrase oxidizes phosphoglucomic acid. We are more interested, however, in the activities of certain holodehydrases containing cozymase, which probably play an essential part in fermentation. One of these is called alcohol dehydrase. It consists of an apodehydrase, which has been separated from yeast and purified as a crystalline protein (p. 23), and of cozymase. The apodehydrase activates hydrogen in the ethyl alcohol, which is oxidized to acetaldehyde while the cozymase is reduced. The reaction is reversible, *p*H and other conditions determining the position of equilibrium.

$$C_2H_5OH + \text{cozymase} \overset{\text{alcohol apodehydrase}}{\rightleftarrows} CH_3CHO + \text{dihydrocozymase} \quad . \quad . \quad (1)$$

A different specific proteinaceous apodehydrase is associated with cozymase in triosephosphate dehydrase, which catalyzes the oxidation of phosphoglycericaldehyde to phosphoglyceric acid.

$$CH_2OH_2PO_3.CHOH.CHO + cz + H_2O \overset{\text{triosephosphate apodehydrase}}{\rightleftarrows} CH_2OH_2PO_3.CHOH.COOH + czH_2 \quad . \quad . \quad (2)$$

There is reason to believe that while fermentation is in progress, reactions (1) and (2) are coupled. Phosphoglyceric acid is produced, with the reduction of cozymase, before acetaldehyde arises from the decarboxylation of pyruvic acid (p. 57); and alcohol dehydrase acts in the reverse way to that to which it owes its name, reducing acetaldehyde to ethyl alcohol, and oxidizing dihydrocozymase to cozymase, which enters again into reaction (2).

It is evident that oxidation of reduced codehydrases I and II is essential if the proper substrates of holodehydrases are to undergo continuous dehydrogenation; and special interest attaches to linkages with systems (*e.g.*, cytochrome systems) that are concerned with oxygen uptake by aerobic organisms. Linkage with systems containing flavin will be considered in the next section. Here we note that neither dihydrocozymase nor dihydrocodehydrase II is autoxidizable. They are, indeed, but slowly

oxidized by methylene blue or cytochrome, suggesting that each of the codehydrases has a relatively high affinity for hydrogen. This affinity can, apparently, be weakened by a specific catalyst, called *coenzyme factor* by Dewan and Green (*74*), and *diaphorase* by other workers (see *e.g.*, Lockhart, *183*), and the reduced codehydrases may then be dehydrogenated by cytochrome. Accordingly, continuous oxidation of the substrate of a holodehydrase containing cozymase would then occur either anaerobically in the presence of methylene blue, or aerobically with oxygen uptake in the presence of cytochrome oxidase.

$$AH_2 + cz \xrightarrow{\text{apodehydrase}} A + czH_2.$$

$$CzH_2 + \text{cytochrome} \xrightarrow{\text{coenzyme factor}} cz + \text{reduced cytochrome}.$$

$$\text{Reduced cytochrome} + O_2 \xrightarrow{\text{cytochrome oxidase}} \text{cytochrome} + H_2O_2.$$

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2.$$

It will be observed that the complete oxidation may be represented by the equation,

$$AH_2 + 1/2\ O_2 = A + H_2O,$$

and that the participating catalysts are all unchanged at the end of the reaction.

Yellow enzyme (flavoprotein dehydrase). Warburg and Christian obtained from yeast a yellow enzyme, which catalyzed certain oxidations, and is now known to be widely distributed in plants and animals. Thanks to the analytical and synthetical work of Kuhn, Theorell, and others, it is established that this holodehydrase (flavoprotein) consists of a proteinaceous apodehydrase combined with alloxazine nucleotide (riboflavin phosphoric acid, see p. 444). When oxidations are in progress the nitrogen atoms of this prosthetic group function in the transport of hydrogen. The dihydropyridine nucleotides (*i.e.*, reduced cozymase and reduced codehydrase II) appear to be the specific reaction-partners of the yellow enzyme, donating to this enzyme hydrogen, which wanders from the nitrogen of the pyridine ring to that of the isoalloxazine ring. The flavoprotein is thereby reduced, and the yellow colour tends to disappear. The leucoenzyme (*i.e.*, dihydroflavoprotein, containing now dihydroalloxazine nucleotide) is autoxidizable, but appears to be more rapidly oxidized by cytochrome than by molecular oxygen. It is also rapidly oxidized by methylene blue or by the complete cytochrome system in the presence of oxygen. Rates of oxidation may therefore be determined anaerobically by Thunberg's technique, or aerobically by measuring oxygen uptake in the Barcroft-Warburg apparatus (p. 263). Moreover, flavoproteins show absorption bands in the visible spectrum, which shift as the state of oxidation changes. Special photoelectric methods have been developed for measuring these changes.

The action of yellow enzyme may be illustrated by the aerobic oxidation of formic acid by an enzyme system containing formic apodehydrase, which Adler and Sreenivasaya (*1*) separated from pea meal. In the presence of cozymase the holodehydrase was reconstituted ; oxygen was absorbed, and the formic acid dehydrogenated with the production of carbon dioxide and water. The enzyme preparation contained flavin compounds. It was found, however, that the rate of oxidation was greatly increased by the addition of flavoprotein. These facts strongly suggest

that flavins, possibly as yellow enzyme, participate in the oxidation. The whole oxidation was found to proceed according to the equation

$$HCOOH + 1/2\ O_2 = CO_2 + H_2O.$$

The experimental evidence suggested that the intermediate stages were

$$HCOOH \xrightarrow{\text{formic dehydrase}} + cz = CO_2 + czH_2.$$

$$CzH_2 + \text{flavoprotein} = cz + \text{dihydroflavoprotein}.$$

$$\text{Dihydroflavoprotein} + O_2 = \text{flavoprotein} + H_2O_2.$$

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2\ O_2.$$

Glutathione. "The first hint that intermediate hydrogen transport might be a process proper to living tissues" appears to have come from the results of experiments in which glutathione, isolated by Sir Frederick Hopkins from yeast, acted as an intermediate carrier. The thiol group in this tripeptide (p. 448) can exist in a reduced form (G-SH) or in an oxidized form (G-S-S-G). Oxidized glutathione may act as acceptor of labile hydrogen transferred from a donator AH_2. The reduced form is oxidized by molecular oxygen in the presence of iron salts. Clearly, the continuous oxidation of a substrate AH_2 is possible in systems containing glutathione. Hopkins and Morgan (*141*) have isolated glutathione from broad bean seeds and young vegetable marrow. From 1,400 gm. of beans they obtained 0·38 gm. of a crystalline specimen. Possible functions of glutathione are mentioned on pp. 42 and 49.

Ascorbic acid (p. 424) and ascorbic oxidase. Vitamin C probably occurs in all growing and in many fully grown cells of chlorophyllous plants, including brown and red algæ. Szent-Györgyi gave the name hexuronic acid to a crystalline preparation, having antiscorbutic properties, which he obtained from cabbages and other sources. This acid is now called ascorbic acid. Its constitution has been verified by synthesis. It is a reducing agent, and can be oxidized to yield dehydroascorbic acid (oxidized form of vitamin C). This oxidation can be reversed to regenerate ascorbic acid (reduced form of vitamin C). It has generally been reported that this vitamin exists in plant cells predominantly in the reduced form, but experiments on the apple (see below) suggest that the relative amounts of the two forms may change during development.

The vitamin C content of most of the edible fruits and vegetables has been measured. The numbers in brackets after the name of the edible organ will give some idea of the range in vitamin C concentration for that organ, expressed in milligrams per 100 gm. fresh-weight tissue : fresh peas (5 to 40), dried peas (zero to 3), sprouting peas (10 to 100), potato (11 to 34) water cress (24 to 75), cabbage (20 to 124), citrus fruits (20 to 100), black currant (90 to 340). Isaac obtained the high value of from 900 to 1,100 for the skin of the South African guava ; the value for the flesh was 300 to 500. Some of these values may be too high, since it has more recently been found out that extraneous reducing substances have in some instances contributed to the apparent vitamin C values. Wokes *et al.* (*336*) reported that young walnuts contain no vitamin C, but give an apparent value of more than 1,000. Unless this is an exceptional result, there must be serious errors in some of the published values for the vitamin C content of plant foods. Many of these values, however, appear to be trustworthy, since chemical analyses were checked by biological assays, in which effects on growing animals were measured. Furthermore, the extraneous reducers have sometimes been separately determined, and allowances made for them. Inasmuch as the maximum amounts of them found by Jackson,

working at Newcastle from 1942–44 with Professor Heslop Harrison, were always relatively small, it is probable that his estimations on Northumbrian rose hips (350 to 1,700), and the range of values (10 to 1,870) reported earlier by Pyke and Melville for the rose hips of the British Isles, accurately represent ascorbic acid variation in these fruits, which have been so much used in recent years as a source of vitamin C. In spite of the possible errors in measurement, it seems safe to conclude that ascorbic acid is not a trace accumulant, but may reach considerable concentrations. We may emphasize this conclusion by noting that in, for example, the lemon, the fraction (ascorbic acid)/(total sugar) may have a value of about 1/20.

Ray (*244*) reported that excised pea embryos synthesized ascorbic acid when placed on ten per cent. gelatin containing certain hexoses or disaccharides, but the metabolic changes by which ascorbic acid is produced from carbohydrates have not yet been elucidated. Extending earlier work (see p. 43), West and Zilva (*331*) obtained evidence that young Bramley's Seedling apples stored at 3° Centigrade synthesized vitamin C. They referred to the possible existence in the fruit on picking of a "chemically related precursor." Presumably the ultimate source is a sugar. It is not known whether the oxidized or the reduced form is produced first.

Nor do we know whether either of these forms is consumed in normal metabolism. The possibility that vitamin C is an intermediate metabolite, and, indeed, has several functions in plant cells, cannot be excluded. Here we shall consider the evidence for Szent-Györgyi's hypothesis that it is in some way concerned with the transfer of hydrogen.

(i.) *Ascorbic acid as a hydrogen donator.* Szent-Györgyi's researches on hexuronic acid (now called ascorbic acid) developed from his discovery that cabbage juice has reducing properties. He also showed that there is in cabbage an enzyme, hexoxidase (now called ascorbic oxidase), which oxidizes the acid aerobically. There are other biochemical methods by which this oxidation can be brought about *in vitro, e.g.*, by the action of orthoquinones produced in a catechol oxidase reaction, and by substances produced in reactions which involve peroxidases. Some of these may function *in vivo* especially when ascorbic acid occurs without its specific oxidase. In contradistinction to biochemical oxidation, autoxidation is effected by molecular oxygen in the presence of copper or iron salts. Chemical oxidation at *p*H values less than 4·2 (see p. 424) by iodine and by special reagents such as 2, 6-dichlorophenol-indophenol have been most useful in vitamin C assays (see below). A general equation for all these oxidoreductions is given in symbols on p. 424 and is here written in words

$$\text{Ascorbic acid} + \text{A} = \text{dehydroascorbic acid} + \text{AH}_2.$$

The dehydroascorbic acid may be reduced chemically, and, probably also biochemically, to regenerate ascorbic acid, but it has never been proved that the above reaction is strictly reversible, *i.e.*, controlled by a single enzyme or catalyst. We note that under certain conditions dehydroascorbic acid may be decomposed by further oxidation. This would mean the irretrievable loss of vitamin C properties. The art of dehydrating cabbages, and other vegetables and fruits, lies in preserving their vitamins. By mere drying, bulk could equally well be made less, calorific and mineral salt values maintained, and attacks by moulds and bacteria warded off. In dehydration, quick scalding by steam inactivates all the oxidation catalysts, but there may remain thermostable oxidizers. Accordingly, the scalded product, after drying in a stream of hot air, is packed in tins filled with nitrogen. It has been reported that when air does not leak

into tins containing dehydrated cabbage, the loss in vitamin C is inconsiderable.

We shall confine our further discussion to the reaction summarized by the above equation when it is controlled by the ascorbic oxidase, and the hydrogen acceptor (A) is oxygen. In 1935, Tauber *et al.* reported that in the giant pumpkin there occurs an enzyme which acts directly on pure ascorbic acid. Oxygen is absorbed and dehydroascorbic acid accumulates. No hydrogen peroxide is produced in this reaction. The enzyme is probably identical with Szent-Györgyi's hexoxidase. It may be widely distributed in plants. For example, James and Cragg (*154*) found that oxygen uptake by juice expressed from week-old etiolated barley shoots was considerably augmented by the addition of ascorbic acid. The juice did not contain catechol oxidase. It did contain flavones and peroxidase, which, acting together in the presence of hydrogen peroxide, can oxidize ascorbic acid; but tests for the presence of hydrogen peroxide always gave negative results. Based on this evidence, the conclusion drawn, that barley seedlings contain ascorbic oxidase, although not indisputable, is highly reasonable. The fact that ascorbic acid is believed to occur in some plants that do not contain its specific oxidase should be remembered.

The activity of ascorbic oxidase may be measured by determining the rate of oxygen uptake when enzyme and ascorbic acid are mixed in some form of the Barcroft-Warburg apparatus, filled with pure oxygen. Tillmans' method, designed for vitamin C assays, may also be used. Ascorbic acid, by reducing, decolorizes aqueous solutions of the blue redox-indicator, 2, 6-dichlorophenol-indophenol, which may therefore be standardized against pure ascorbic acid. This standard indicator may then be used to determine the amount of ascorbic acid remaining unoxidized after ascorbic acid oxidase has acted for a period on a known weight of pure ascorbic acid. The amount of ascorbic acid oxidized is calculated by difference, and is a measure of oxidase activity.

James and Cragg (*154*) found that the enzyme which oxidized ascorbic acid when added to barley juice was completely inhibited by M/1000 cyanide. According to Sumner and Somers (*298*) ascorbic oxidase is inhibited by hydrogen cyanide, but not by hydrogen sulphide or carbon monoxide, and they note reports that a purified enzyme from squash, *Cucurbita pepo condensa*, had a bluish colour and contained 0·15 per cent. copper, and that another from cucumber contained 0·25 per cent. copper. The presence of copper would account for the enzyme's sensitivity to cyanide. It still remains to be proved, however, that all the oxidases which appear to be specific for ascorbic acid are copper proteids (see remarks about catechol oxidase on p. 29).

(ii.) *Dehydroascorbic acid as a hydrogen acceptor.* It is noteworthy that in spite of the presence in cells of ascorbic oxidase, or of some other system (*e.g.*, catechol oxidase) that can oxidize ascorbic acid, this substance occurs mainly in the reduced state. Conceivably oxidizing enzyme and substrate are kept apart in healthy cells (see remarks about oxidases on pp. 29 and 70). It is possible that ascorbic acid is chemically protected from attack. Sir Frederick Hopkins and Morgan obtained evidence that in cabbage and cauliflower there is an enzymic mechanism which enables reduced glutathione to oppose the oxidation of ascorbic acid. The glutathione was added to the reacting system. Crook and Morgan (*65*) have developed this research and appraised the facts so far discovered. The enzyme which is effective in the presence of glutathione is distinct from ascorbic oxidase. They call it *dehydroascorbic acid reductase*. Its distribution is

moderately wide. It was absent in only eight out of the thirty species investigated. " Addition of the juice from nine species at least doubled the rate of reduction of dehydroascorbic acid caused by GSH alone without added enzyme. The most active species were cauliflower and broad bean, in both of which glutathione will protect ascorbic acid from oxidation by atmospheric O_2." Some evidence was cited that species (*e.g.*, Hydrangea) lacking the enzyme can, nevertheless, reduce dehydroascorbic acid.

Much interest attaches to the possibility that *in vivo* dehydroascorbic acid may be reduced, either spontaneously or under the agency of an enzyme, by some metabolically produced hydrogen donator (*e.g.*, dihydrocozymase) to yield ascorbic acid. The equation for such a reduction may be written thus :—

$$\text{Dehydroascorbic acid} + BH_2 = \text{Ascorbic acid} + B$$

For the ascorbic acid system to act continuously in cellular oxidoreductions a reduction of this kind must occur. We note that chemical reduction is easily effected by hydrogen sulphide.

The conclusions drawn by Zilva, Kidd, and West (*339*), imply that reduction takes place as apple fruits growing on a tree approach maturity. They state that both ascorbic and dehydroascorbic acid are always present in the apple, that the concentration of (ascorbic acid + dehydroascorbic acid) remains constant throughout the growth of the fruit (*i.e.*, vitamin C is synthesized in amounts proportional to fresh weight increase), but that, as an apple approaches maturity, the concentration of dehydroascorbic acid decreases while that of ascorbic acid increases. James, Heard, and James (*155*), state that dehydrogenase systems (for example, lactic acid dehydrogenase, triosephosphate dehydrogenase) may be linked with the (ascorbic acid/ascorbic acid oxidase/oxygen) system in barley. There were so many components in the mixtures investigated at Oxford that rigid proof was not obtained, but some of the evidence was very suggestive. James and Cragg (*154*) reported that the oxygen uptake of (barley juice + ascorbic acid), which was in part attributable to the activity of the ascorbic acid oxidase in the juice, was augmented by the addition of lactate, and it was proved that a small amount of pyruvate accumulated. A possible interpretation is that hydrogen is transferred to dehydroascorbic acid from lactic acid, activated by its dehydrogenase. ($CH_3.CHOH.COOH$ + dehydrogenase) + dehydroascorbic acid = $CH_3.CO.COOH$ + ascorbic acid.

The ascorbic acid is then oxidized, with oxygen uptake, by the ascorbic acid oxidase, and *the dehydroascorbic acid is thereby regenerated.* We emphasize this point because it is possible that in the past too much attention has been paid to the fact that vitamin C occurs in plants mainly in the reduced state, and to finding out what work ascorbic acid does as a hydrogen donator. Instead, it may be that vitamin C functions in plant cells as the hydrogen acceptor, dehydroascorbic acid, which may therefore resemble, functionally, the orthoquinones produced by the catechol-oxidase system. The fact that the vitamin accumulates mainly in the reduced state (*cf.* the accumulation of catechol compounds rather than orthoquinones in plant cells) may simply mean that the reducing systems producing ascorbic acid are more active than the enzymes which oxidize it. On these matters, however, we must wait for further evidence, critically considered by specialists in this field of study.

James *et al.* (*155*) obtained evidence that barley juice contains enzymes which can convert hexose diphosphate into phosphoglycericaldehyde, and oxidize this triosephosphate to yield phosphoglycerate (see p. 56, reactions iv to viii). If we accept this evidence, we may infer that barley juice

contains the enzyme triosephosphate dehydrogenase, which is known to require cozymase (codehydrase I) as a hydrogen acceptor (p. 57). What these investigators claim to have demonstrated will be recorded in their words: "In manometric experiments, clarified saps with hexosediphosphate absorbed little or no O_2. On addition of ascorbic acid, rapid O_2 uptake occurred in excess of the oxygen uptake caused by ascorbic oxidase alone. Addition of coenzyme I to sap with ascorbic acid caused a large increase of O_2 uptake, far beyond the O_2 equivalent of the coenzyme added. Hexosediphosphate still further increased this O_2 uptake." The reasonable hypothesis put forward was that triosephosphate, activated by its dehydrogenase, donates hydrogen to cozymase, which thereupon becomes reduced to dihydrocozymase (p. 57, reaction vii). For the reaction to go on until all the substrate (ultimately hexose diphosphate) is consumed there must be a mechanism for the regeneration of cozymase. The work at Oxford on barley juice has led to the important conclusion, expressed in other words by the investigators, that this regeneration is brought about by the transfer of hydrogen from dihydrocozymase to dehydroascorbic acid, produced by the oxidation, with oxygen uptake, of reduced ascorbic acid under the agency of ascorbic acid oxidase.

$$CzH_2 + \text{dehydroascorbic acid} = cz + \text{ascorbic acid.}$$

Reconsidering the earlier work on lactic acid dehydrogenase, James *et al.* point out that this enzyme in yeast may be able to act without cozymase, but that animal dehydrogenase requires cozymase. Accordingly they think it is possible, but not certain, that the link between the lactic acid dehydrogenase and the ascorbic acid system in barley may be through cozymase. Evidently the above equation implies that any system producing dihydrocozymase in cells (and several are known) may produce ascorbic acid (the normally occurring form of vitamin C) from dehydroascorbic acid, which can be so readily formed *in vitro* by oxidation systems of aerobic plants (sub-section (i.) above).

This work at Oxford has furnished the best evidence so far in favour of Szent-Györgyi's original suggestion that in green plants his (hexuronic acid/hexoxidase/oxygen) system has a redox function comparable to that of the [(reduced cytochrome)/cytochrome oxidase/oxygen] system in animal cells and yeast. We point out, however, that the concentration of ascorbic acid in plant cells may be much higher than that of cytochrome, which, like most of the other intermediate carriers, is a trace accumulant in living cells.

Di- and tricarboxylic acids and oxidoreductions. Evidence has been obtained of the occurrence in animal cells, bacteria, and yeast, of enzymes that catalyze the oxidoreduction and, therefore, among other events, the interconversion of some di- and tricarboxylic acids. So far investigations on the higher plants have been few, in spite of the fact that acids may attain high concentrations in some of them (see chap. XV).

(i.) *Enzymes acting in the metabolism of C_4 acids.* The dehydrogenation of succinic acid to yield fumaric acid is controlled by *succinic dehydrogenase*, which is linked with the cytochrome system (p. 37). It should be noted that this dehydrogenation is specifically inhibited by malonic acid in concentrations of 0·001 to 0·005 molar. If a cellular oxidation is inhibited by malonic acid, the inference drawn is that the succinic dehydrogenase is a component of the oxidation system (Krebs, *176*). *Fumarase* governs the reversible hydration-dehydration reaction between fumaric acid and malic acid.

$$HOOC.CH{=}CH.COOH + H_2O \underset{\text{fumarase}}{\rightleftarrows} HOOC.CHOH.CH_2.COOH$$

In the presence of *malic dehydrogenase*, which may require coenzyme I malic acid is oxidized to oxalacetic acid. The reaction is reversible.

$$HOOC.CHOH.CH_2.COOH \underset{\text{malic dehydrogenase}}{\rightleftharpoons} HOOC.CH_2.CO.COOH$$

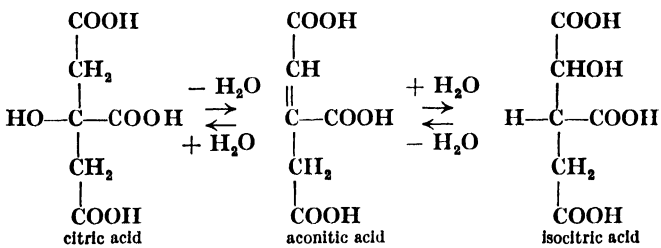
Oxalacetic acid has recently been enzymically decarboxylated with the production of pyruvic acid and carbon dioxide (see p. 338). There is some evidence that the reaction is reversible.

$$HOOC.CH_2.CO.COOH \rightleftharpoons CH_3.CO.COOH + CO_2$$

A biochemical discovery of high importance is Wood and Werkman's that dicarboxylic acids containing four carbon atoms can be synthesized by the union of a C_3 compound, which is probably pyruvic acid or a phospho derivative thereof, and carbon dioxide, absorbed from the outside or coming from some parallel metabolic reaction.

(ii.) *Enzymes acting in the metabolism of C_6 acids.* Citric acid is consumed by some organs of the higher plants (p. 320) and by mould fungi (p. 336), but the products have not been identified. Wagner-Jauregg and Rauen (see Krebs, *176*) demonstrated the presence in cucumber seeds of a *citric dehydrogenase*, which oxidized citric acid with the absorption of oxygen and the liberation of carbon dioxide. Isocitric acid was oxidized equally rapidly. The products were not identified. When liver tissue oxidizes citric acid, α-ketoglutaric acid is produced. This important discovery was made by differentially inhibiting with arsenite the enzyme which decomposes α-ketoglutaric acid, and then identifying the accumulating acid as its 2 : 4 dinitrophenylhydrazone.

Citric acid dehydrogenase is a mixture of enzymes. *Aconitase*, which may be resolvable into two components, governs the equilibria represented by the following equations :—



Isocitric dehydrogenase, which occurs in animal tissues, converts isocitric acid into α-ketoglutaric acid ($HOOC.CO.CH_2.CH_2.COOH$) and carbon dioxide, *i.e.*, there exists an enzymic mechanism for converting tricarboxylic acids into dicarboxylic acids. Aconitase has been detected in wheat, rye, peas, and soya beans. It remains to be proved that isocitric dehydrogenase occurs with it.

In animal tissues succinic acid can come from α-ketoglutaric acid. In the change carbon dioxide is given off and oxygen absorbed. The reverse reaction has not been demonstrated.

If these reactions occur in plants (see fig. 1), four acids (succinic, fumaric, malic, oxalacetic) could be produced by the decomposition of citric acid. There are possible side reactions causing the formation of the amino acids, aspartic and glutamic acids, and their corresponding amides. Further, pyruvic acid might be produced by the decarboxylation of oxalacetic acid. Pyruvic acid can yield acetaldehyde (p. 59), lactic acid

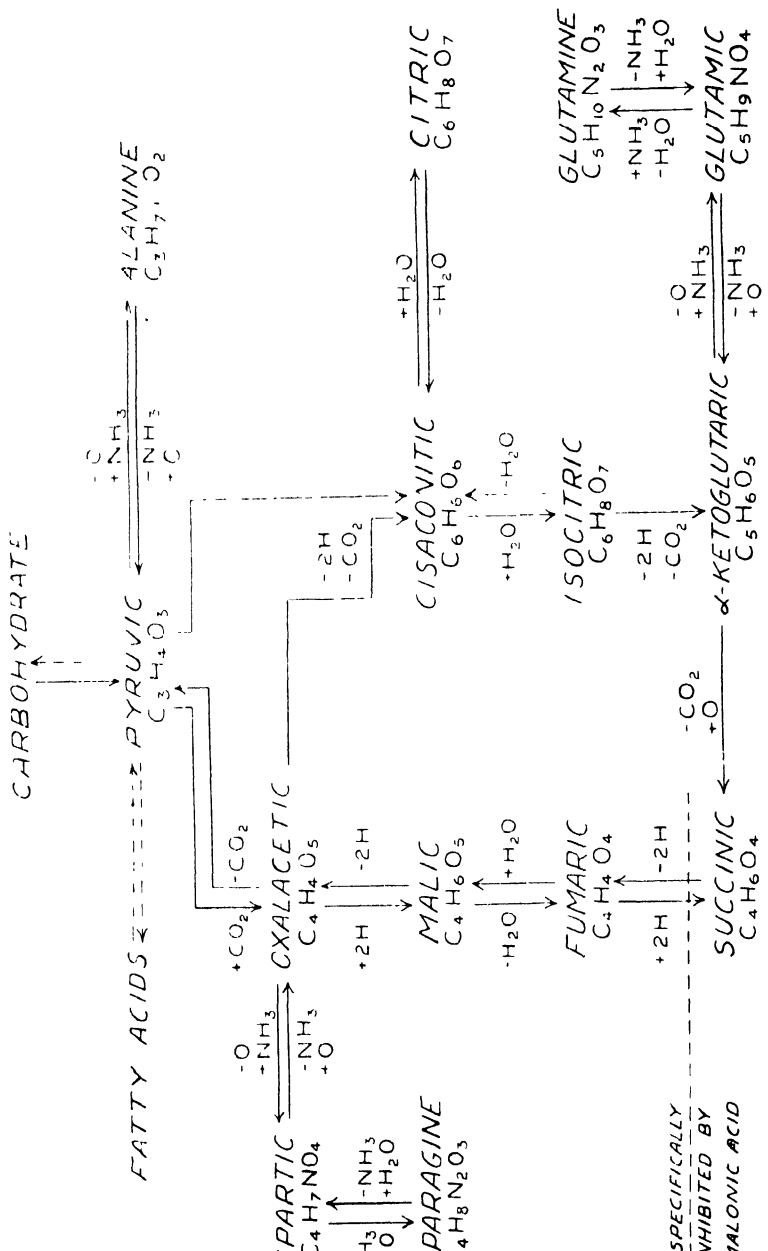


FIG. 1. Possible metabolic changes involving di- and tricarboxylic acids. (See text in this subsection, the next subsection, pp. 44 to 49, and chapter XV.)

(p. 55), alanine (p. 48), among other products. Its conversion into carbohydrate is not a rejected possibility.

There remains one of the reactions shown in fig. 1 which we have not mentioned, viz., the production of C_6 tricarboxylic acids with branched chains. Krebs suggested that they might arise by the union of pyruvic and oxalacetic acids. This union has since been actuated by an enzyme *citrogenase*, which occurs in kidney (p. 339). The precise biochemical mechanism is still under consideration. Werkman and Wood (*329*) give reasons for assuming that cisaconitic acid is produced by the condensation of the enol form of oxalacetic acid with pyruvic acid. The reaction involves decarboxylation and oxidation by dehydrogenation. The identity of the hydrogen acceptor (A) is not stated.

$$\begin{array}{c} HOOC.C(OH)=CH.COOH + CH_3.CO.COOH + A= \\ HOOC.CH=C.COOH + CO_2 + AH_2 \\ \quad\quad\quad\

donators, and oxalacetic and fumaric acids hydrogen acceptors. These acids may serve in these capacities when they are present in cells in which are occurring oxidoreductions very different from those illustrated in fig. 1. Indeed, the tricarboxylic acid cycle was proposed by Krebs (*176*) as a modification of Szent-Györgyi's earlier hypothesis to indicate how C_4 and C_6 acids, existing in low concentrations in animal tissues, may function as catalysts. First Szent-Györgyi, and then others, obtained evidence that small amounts of any of the C_4 acids, when added to animal tissue, may catalyze its oxygen uptake. It has been reported that the respiratory quotient is not changed, and that one of the substrates involved in this oxidation is triosephosphate, which is dehydrogenated to yield phosphoglycerate, when cozymase is present as a hydrogen acceptor. Evidence exists, therefore, that some of the acids mentioned in fig. 1 can act as catalysts in carbohydrate metabolism, but whether they actually do so serve is still controversial.

Amino acid oxidases, with incidental reference to aminases and transaminases. Evidence is cited by Chibnall (*59*) and by Sumner and Somers (*loc. cit.*) that naturally occurring amino acids, when activated by their dehydrogenases (some of which are composed of apodehydrase plus codehydrase), can undergo oxidative deamination with the formation of α-imino acids.

$$R.CHNH_2.COOH + \text{codehydrase} \overset{\text{apodehydrase}}{\underset{+\text{ dihydrocodehydrase}}{\rightleftarrows}} R.C{=}NH.COOH$$

Spontaneous hydrolytic deamination of an α-imino acid yields an α-keto acid.

$$R.C{=}NH.COOH + H_2O \rightleftarrows R.CO.COOH + NH_3$$

The process is continuous when it is coupled with another in which dihydrocodehydrase is oxidized. *In vivo* this may lead to the absorption of oxygen. The reactions are reversible. This means that they may play a part in living cells not only in the production from protein of nitrogen-free compounds such as the vegetable acids or, if pyruvic acid is the α-keto acid formed (see p. 64), the carbohydrates, but also in the synthesis of protein from acids or carbohydrates. For example, the amination of pyruvic acid would yield alanine (see fig. 1).

Von Euler *et al.* in 1938 achieved the first enzymic synthesis of an amino acid from a keto acid and ammonia in a cell-free system when they proved that glutamic acid is produced from α-ketoglutaric acid and ammonia in the presence of animal apodehydrase and either codehydrase I or codehydrase II. The highest activity was shown by glutamic dehydrogenase from mammalian liver. The power that plants have of synthesizing amino acids is far more extensive than that possessed by animals. In von Euler's success, there is, therefore, a challenge to plant biochemists to learn the art of separating from plant cells systems that will synthesize amino acids *in vitro*.

Glutamic acid and aspartic acid are key substances in plant metabolism. Damodaran and Nair have obtained from leguminous seedlings a *glutamic dehydrogenase* which acts oxidatively on glutamic acid with the production of ammonia and α-ketoglutaric acid ($COOH.CO.CH_2.CH_2.COOH$). Chibnall holds that it is by the reversal of this process that glutamic acid, and, subsequently, under the agency of glutaminase (p. 27), the well-known amide glutamine, are synthesized in plants (see fig. 1). Indeed, by injecting leaves of perennial rye grass with ammonium α-glutarate (for a hint about the method see p. 154), he has caused them to make glutamine.

We have no knowledge of the action *in vitro* of an aspartic acid dehydrogenase, which should catalyze the reversible transformation of aspartic acid into oxalacetic acid and ammonia. This is one of the reactions postulated in fig. 1. Aspartic acid synthesized thus could be converted into asparagine by asparaginase (p. 27). There is an oxidative deaminase, *aspartase*, discovered in *Bacillus coli* by Quastel and Woolf, which attacks aspartic acid with the production of fumaric acid and ammonia.

$$COOH.CH_2.CHNH_2.COOH \rightleftarrows COOH.CH=CH.COOH + NH_3$$

The reaction is reversible. Weak aspartase activity has been reported in germinating peas, the leaves of young red clover, and other plant organs.

The process of transamination probably plays an important part in metabolism (*cf.* transphosphorylation, p. 53). *Transaminase* (or *aminopherase*), which is said to occur in practically all animal tissues, acts on certain amino acids in the presence of α-keto acids, with the result that the α-keto acid becomes aminated and the amino acid deaminated. The existing evidence suggests that one of each pair of interacting acids must be dicarboxylic. Virtanen and Laine have reported that there is a transaminase in crushed pea plants, in the presence of which aspartic acid and pyruvic acid interact with the production of oxalacetic acid and alanine.

$$COOH.CHNH_2.CH_2.COOH + CH_3.CO.COOH \rightleftarrows COOH.CO.CH_2.COOH + CH_3.CHNH_2.COOH$$

According to von Euler, glutamic acid also participates in transaminations in the higher plants. Chibnall sees in this exchange mechanism one possible method by which asparagine and glutamine may, after deamidation (p. 204), be used in amino acid, and, therefore, in protein synthesis. He considers that this mechanism may be particularly important when extensive interconversion of amino acids must occur because the amino acid composition of the proteins at a sink (*e.g.*, meristem, developing storage tissue) differs widely from that of proteins at a source (*e.g.*, green leaves, storage tissue from which food is being translocated).

Mutases. The name mutase is given to an enzyme which catalyzes a hydrolytic oxidoreduction known as a dismutation (p. 418). Acetaldehyde mutases obtained from liver, yeast, and certain bacteria, have been intensively studied (Dixon, *78*). It is uncertain whether an aldehyde mutase is a single enzyme or consists of two linked dehydrogenases, but it has been shown that cozymase is an essential component of the reacting system. The following stages may occur in the dismutation of acetaldehyde under mutase influence :—

$$CH_3CHO + H_2O + cz = CH_3COOH + czH_2$$
$$CH_3CHO + czH_2 = C_2H_5OH + cz.$$

Hopkins and Morgan (*141*) have reported that *glyoxalase* is widely distributed in plants. In the presence of glutathione it converts methyl glyoxal into lactic acid,

$$CH_3.CO.CHO + H_2O = CH_3CHOH.COOH.$$

E. The Zymase Complex and Fermentation

Components of the reacting system. The substrate and end products of alcoholic fermentation, whether by the yeast organism or by zymase (see p. 21), are described by Gay-Lussac's equation,

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

The hexose may be *d*-glucose, *d*-fructose, or *d*-mannose (see p. 430), but *d*-galactose is not fermented.

Products, such as the so-called fusel oils, which accumulate when fermentation is carried out by growing yeast, do not concern us here, since they result from the metabolism of proteins and other substrates in the living organisms, and never arise from zymase cleavage *in vivo* or *in vitro*.

Harden and Young (see Harden, *117*) discovered that zymase in cell-free juice prepared by Buchner's method is compounded of a thermolabile part, now called apozymase, and a thermostable diffusible organic substance (cozymase), which they separated from apozymase by ultrafiltration through a gelatine filter. Hexokinase, phosphohexoisomerase, zymohexase, phosphoglyceroisomerase, triosephosphatedehydrogenase, phosphoglyceromutase, enolase, carboxylase, and alcohol dehydrogenase, are now known to be among the distinct specific enzymes present in the apozymase complex. This is a formidable list, and is probably not a complete one. Furthermore, at least three coenzymes play essential parts. There is Harden and Young's cozymase, whose chemical constitution has now been established by synthesis (p. 444), and there are the cophosphorylases (p. 53) and cocarboxylase (p. 60). Harden and Young also discovered that the continued activity of zymase depends upon the presence of inorganic phosphate in the reacting medium. More recently, Lohmann has shown that magnesium ions are essential.

There is no standard method of preparing a pure zymase complex of invariable composition. Not only may the relative proportions and activities of the essential enzymic and coenzymic components vary, but other enzymes (*e.g.*, phosphatases, lactic acid dehydrogenase) may be present as impurities. It is not surprising, therefore, that in experiments on fermentation *in vitro* many side reactions have been observed in addition to the primary reactions. With the disappearance of sugar there may accumulate not only ethyl alcohol and carbon dioxide, but small amounts of the intermediate products of fermentation (*e.g.*, triosephosphates, acetaldehyde) and of the end products (*e.g.*, lactic acid, glycerol, methylglyoxal) of side reactions. Consideration will be confined here to such reactions as are believed to be steps on the way to the production of ethyl alcohol and carbon dioxide. Most of the stages to be described have been observed *in vitro* under control of the single enzyme components of the whole zymase complex, which have been made to do their special work in isolation from the other components or in some other way (see pp. 215–17) forced to reveal their powers. From the partial reactions so studied, schemes for the whole zymase-cleavage process have been constructed; the one now favoured has developed from Meyerhof's. This, in its turn, incorporated certain

indisputable facts (*e.g.*, that ethyl alcohol is produced by the reduction of acetaldehyde, which comes with carbon dioxide from the decarboxylation of pyruvic acid) in Neuberg's theory, which dominated the field in the 1920's.

There is evidence (see, *e.g.*, MacFarlane, *341*) that similar stages are passed through when alcoholic fermentation goes on in the yeast organism living in a nutrient solution containing sugar. For equivalent amounts of yeast and yeast juice, and under conditions parallel in other essential ways, the rate of production of ethyl alcohol and carbon dioxide is higher *in vivo* than *in vitro*. In one experiment reported by Harden (*117*) it was found to be forty times higher. Moreover, certain intermediate products (*e.g.*, hexosephosphates) do not accumulate to the same extent (MacFarlane, *loc. cit.*). This may be attributed to the superior organization of chemical events in the living cell, where the cyclical changes that we shall describe are far more effectively controlled and co-ordinated than they are in systems working *in vitro*. Since the whole zymase system is so complex it is surprising that yeast press juice and zymin, products obtained by very rough treatment of yeast cells (p. 21), ever show even feeble powers of breaking sugars down all the way to ethyl alcohol and carbon dioxide. It is a remarkable fact that these powers are often far from feeble. For example, in one experiment 3 c.c. of fermentative carbon dioxide were produced in five minutes by the action of 25 c.c. of yeast juice coming from 36 gm. of pressed yeast. In another experiment a maximum CO_2 output of 14 c.c. in five minutes was found when zymin fermented fructose in the presence of inorganic phosphate. The inference must surely be that not only were all the enzymes, coenzymes and activating ions present in the yeast juice and zymin, but their structural relations to one another, in spite of the drastic preparative processes, were still sufficiently a reflection of those existing in the protoplasm of the yeast cell for the chemical events of the fermentation *in vitro* to be qualitatively, although admittedly not quantitatively, identical with those occurring *in vivo*.

It is probable that closely similar, and possibly identical events, take place when ethyl alcohol is produced by other fungi (see Kostyschew, *175*) or by the higher plants (p. 302). In vertebrate muscle there is an enzyme complex which differs slightly in composition, but functionally very significantly, from zymase. This muscle enzyme cleaves sugar derived from glycogen (p. 61) with the production of pyruvic acid by the same stages as those passed through in zymase cleavage. In muscle, however, the pyruvic acid is reduced to lactic acid (see fig. 2). The use of the term *glycolysis* is confined by some animal biochemists to this production of lactic acid from carbohydrates, but many physiologists have employed it, as we shall

continue to use it in this book, for any form of sugar cleavage. In the growth in the 1930's of our knowledge of phosphorylative glycolysis, discoveries have sometimes been made in experiments on muscle to be confirmed by others on yeast, and at other times those on yeast have pointed the way. An informative summary with a full bibliography of a fascinating story will be found in Sumner and Somers (*loc. cit.*). To appreciate the brilliance with which very difficult experimental problems have been solved some of the original papers cited in the bibliography must be read.

The partial reactions taking place in alcoholic fermentation are of several types. Broadly, we may state that the passage from C_6 to C_2 compounds is effected (*a*) by preparatory phosphorylations at the C_6 and C_3 stages, (*b*) by glycolytic cleavage to produce triosephosphates, (*c*) by oxidoreductions and transphosphorylations that lead to the production of pyruvic acid, (*d*) by the decarboxylation of this acid with the production of carbon dioxide and acetaldehyde, which is then reduced to ethyl alcohol. Before considering the stages in some detail it will be desirable to give special consideration to the process of phosphorylation, for there is a strong suggestion that ideas put forward as a result of the study of this process in glycolysis may have far-reaching implications in the realm of general physiology.

The nature and functions of the phosphorylations occurring in zymase cleavage. In the experiments by which Harden and Young established that inorganic phosphates accelerate alcoholic fermentation by yeast juice or zymin they also made the discovery that, while fermentation was in progress, the inorganic phosphate esterified the hexose substrate with the production of hexose diphosphate. This reaction also takes place *in vivo* (MacFarlane, *341*), but one result of the destruction of cell organization in the preparation of yeast juice or zymin is that there is an over-phosphorylation of hexose *in vitro*. Accordingly, hexosephosphates accumulate in higher concentrations in enzymic than in organismic fermentations. Harden and Young's discovery gave to biochemistry the foundation idea of the importance of phosphorylations in carbohydrate metabolism, on which much has since been built.

Morgan and Robison (*205*) have shown that the ester of Harden and Young is fructofuranose-1 : 6-diphosphate (p. 430), and that this ester is produced whether one starts with glucose or fructose. Three monophosphoric esters are known, viz., the Cori-ester (glucopyranose-1-phosphate), the Robison ester (glucopyranose-6-phosphate), and the Neuberg ester (fructofuranose-6-phosphate). It should be noted that all these esters are acidic substances. The preparation of their barium and lead salts has played an important part in their identification. Magnesium hexosediphosphate can be bought at the British Drug Houses. Soluble hexosephosphates are easily distinguished

from inorganic phosphates, because they are not precipitated by magnesium citrate mixture or by uranium acetate solution.

A point which has been much emphasized recently (Lipmann, *181*) is that the free energy which is potential in the phosphate bonds of ester phosphates ($R\text{-}O\text{-}PO_3H_2$), *e.g.*, hexosephosphates and triosephosphates, has the relatively low value of about 3,000 cals. In contrast, which may be very significant in cell energetics (p. 261), other phosphate bonds may have the relatively high free energy values of from 8,000 to 11,000 cals. We shall shortly come across two such types, viz., the acyl phosphate linkage ($R.CO \sim PO_3H_2$) in diphosphoglyceric acid, and the enol phosphate linkage

$$(R.CH_2 = CHO \sim PO_3H_2.COOH)$$

in phosphopyruvic acid. Adopting Lipmann's symbols, we shall occasionally represent the ester bond by — ph and the active bond by ~ ph, and we shall assume that their respective energy values are 3,000 and 10,000 cals. on the average.

Special interest centres upon the phosphate bonds in the three substances which have hitherto been classed as cophosphorylases. These substances are adenosine monophosphoric acid (adenylic acid, p. 444), adenosine diphosphoric acid (ADP) and adenosine triphosphoric acid (ATP). Adenylic acid (ad — ph) is a substituted ribose ester with a phosphate bond of low energy value, but ADP (ad — ph ~ ph) has one active bond, and ATP (ad — ph ~ ph ~ ph) has two such bonds. ATP and ADP serve as phosphorylating agents in the production of ester phosphates. Such esterifications are described as exergonic because there occurs during their course a loss of about 7,000 cals. free energy for every ~ ph bond converted into a — ph bond. ATP and ADP are acting as phosphate donators when they are causing such intermolecular transphosphorylations. Since adenylic acid does not contain an active phosphate bond it is not a phosphate donator, but it functions in cell metabolism as a phosphate acceptor, being converted into ADP. For this conversion to occur, since ADP contains one ~ ph bond, an organic phosphate containing an active phosphate linkage (*e.g.*, an acyl phosphate or an enol phosphate) must serve as donator. When ADP is converted into ATP it itself acts as a phosphate acceptor at this high energy level of transphosphorylation.

The phosphorylations that occur before glycolysis, and the phosphorylations and transphosphorylations that occur after glycolysis, constitute what is called the phosphate cycle in fermentation. At a certain stage inorganic phosphate enters into the cycle, and, as a net result of the whole cycle, for every molecule of hexose fermented two active ~ ph bonds become incorporated in ADP or ATP, so raising the phosphate bond energy potential of the whole system. This

energy is ultimately derived from the free energy in the hexose molecule that undergoes glycolysis. It is clear that alcoholic fermentation is not now regarded as a process in which the energy contained in the substrate is completely dissipated. A point that we shall take up again in considering the aerobic catabolism of sugars in the higher plants (p. 306) is that the phosphate cycle in ordinary alcoholic fermentation is completed when pyruvic acid is produced. Lipmann emphasizes the fact that phosphorylative glycolysis not only produces those C_3 and C_2 compounds which may be used in other metabolic events in cells (p. 187), as has for many years been believed to be its main function, but also harnesses in active phosphate bonds some of the free energy, coming from the carbohydrates, for use in cell anabolism. A clear instance is afforded by events in muscle tissue (see fig. 2), where some of the free energy of glycogen is transferred to ATP, which can then donate phosphate to creatine with the production of phosphocreatine (phosphagen). This is an essential reaction in the recovery of fatigued muscle. To what use active phosphate bond energy is put in plant cells is a problem which will doubtless be much discussed in future years.

The partial reactions in alcoholic fermentation. The reactions numbered below by small roman numerals have been separately studied, and are at the present day believed to occur as steps in zymase cleavage, and in the order in which they are recorded here. They are portrayed schematically in fig. 2.

(*a*) *The phosphorylation of hexoses by ATP with the final production of fructofuranose*-1 : 6-*diphosphate.* With either glucose or fructose—we shall not consider mannose—as the substrate the *hexokinase* component of zymase, in the presence of ATP, which acts as a phosphate donator, and magnesium ions, catalyzes the production of the corresponding hexose-6-phosphate and ADP. This is an exergonic transphosphorylation (p. 53).

(i.) $$C_6H_{12}O_6 + ATP \xrightarrow{\text{hexokinase}} C_6H_{11}O_5.OPO_3H_2 + ADP$$

Under the agency of *phosphohexoisomerase* an equilibrated mixture of glucose-6-phosphate (Robison's ester) and fructose-6-phosphate (Neuberg's ester) is formed. Thus, if glucose is the substrate for reaction (i.), Robison's ester is produced first, and this is then partially converted into Neuberg's ester.

(ii.) $$\underset{\text{CHOH}.(\text{CHOH})_3.\text{CH}.\text{CH}_2\text{OPO}_3\text{H}_2}{\overset{\text{phosphohexoisomerase}}{\rightleftarrows}} \underset{\text{CH}_2\text{OH}.\text{COH}.(\text{CHOH})_2.\text{CH}.\text{CH}_2\text{OPO}_3\text{H}_2}{}$$

A further exergonic transphosphorylation then occurs when ATP donates another phosphate group to Neuberg's ester with the production of fructofuranose-1 : 6-diphosphate (Harden and Young's ester).

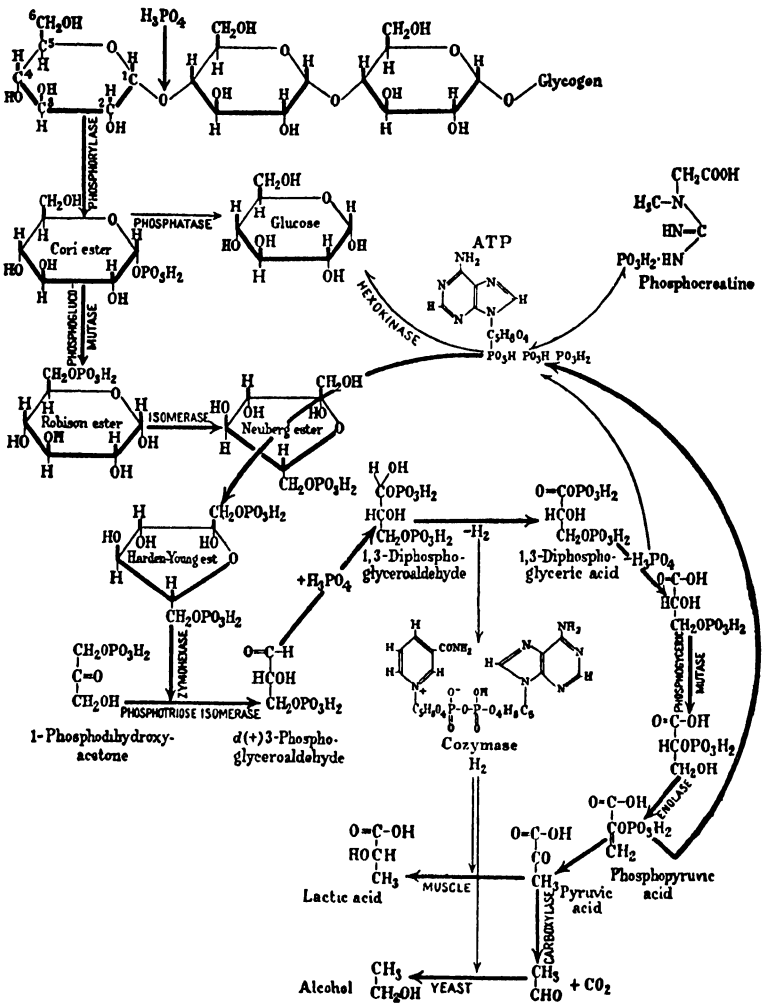


FIG. 2. Schematic representation of the partial reactions in the production from glycogen of ethyl alcohol by yeast and of lactic acid by muscle. [Photographed from figure in Mann and Lutwak-Mann, *138*, and used here by kind permission of these authors.

An enzyme, requiring magnesium ions, appears to be necessary for this reaction. We can call the enzyme *phosphohexokinase* as reactions (i.) and (iii.) are similar.

(iii.) $CH_2OH.COH.(CHOH)_2.CH.CH_2OPO_3H_2 + ATP \overset{\text{phosphohexokinase}}{\rightleftarrows} CH_2OPO_3H_2.COH.(CHOH)_2.CH.CH_2OPO_3H_2 + ADP$

The reactions governed by phosphohexoisomerase and phosphohexokinase are reversible, but it has not yet been proved that reaction (i.) can be reversed under the agency of hexokinase. We note that it has long been known that hexosephosphates can be hydrolytically dephosphorylated by phosphatases (p. 25), *i.e.*, by a mechanism which is different from and not the reverse of the transphosphorylations occurring in zymase cleavage.

(*b*) *The glycolysis of fructofuranose-*1 : 6-*diphosphate with the final production of* 3-*phosphoglycericaldehyde.* An enzyme now generally called *zymohexase* or *aldolase*, which is extremely stable, retaining its activity at 70° C., cleaves hexosediphosphate with the production of dihydroxyacetonephosphate, which is then acted on by another enzyme, *phosphoglyceroisomerase*, to yield (d)-3-phosphoglycericaldehyde. Both reactions are reversible. Meyerhof and Lohmann found that the same equilibrated mixture of the three components was obtained in thirty seconds whether they started with the hexosediphosphate or one of the phosphotrioses.

(iv.) $C_6H_{10}O_4(OPO_3H_2)_2 \overset{\text{zymohexase}}{\rightleftarrows} 2CH_2OPO_3H_2.CO.CH_2OH.$

In the remaining equations we shall represent the fate of a single molecule of dihydroxyacetonephosphate, but we must remember that in constructing an overall equation for alcoholic fermentation (p. 49), two molecules of this substance are involved for every molecule of hexose fermented.

(v.) $CH_2OPO_3H_2.CO.CH_2OH \overset{\text{phosphoglyceroisomerase}}{\rightleftarrows} CH_2OPO_3H_2.CHOH.CHO$

In an isolated system at equilibrium there is only three per cent. of the aldotriosephosphate present, the remaining ninety-seven per cent. being the ketotriosephosphate, but when the whole zymase complex is at work only the aldotriosephosphate is acted on. Accordingly all the dihyroxyacetonephosphate becomes converted into (d)-3-phosphoglycericaldehyde. This aldotriose was synthesized in 1931, and it was soon after shown to be fermentable. Evidence of the production of triosephosphate in zymase cleavage came from experiments in which hexosediphosphate was attacked in the presence of sodium iodoacetate. The reason for this is explained after the description of reaction (vii.) below.

(*c*) *The further phosphorylation by inorganic phosphate of phospho-*

glycericaldehyde, and the production of 1 : 3-*diphosphoglyceric acid by an oxidoreduction with cozymase as hydrogen acceptor.* All the substances mentioned so far have been isolated in experiments on fermentation. In 1939 Negelein and Brömel added 1 : 3-diphosphoglyceric acid to this list. According to Warburg and Christian the first step in the production of this acid is the primary phosphorylation by inorganic phosphate of 3-phosphoglycericaldehyde to give 1 : 3-diphosphoglycericaldehyde, which then serves as the hydrogen donator in the oxidoreduction that follows. Meyerhof believes that the change is enzymic. The diphosphotriose has not yet been isolated.

(vi.) $CH_2OPO_3H_2.CHOH.CHO + H_3PO_4 \overset{\text{enzyme (?)}}{\rightleftarrows} CH_2OPO_3H_2.\overline{C}HOH.CH(OH)(OPO_3H_2).$

The oxidoreduction is governed by the enzyme *triosephosphate dehydrogenase*, which has been prepared as a crystalline protein by Warburg and Christian, and specifically requires cozymase as hydrogen acceptor.

(vii.) $CH_2OPO_3H_2.CHOH.CH(OH)(OPO_3H_2) + cz \underset{\text{triosephosphate dehydrogenase}}{\rightleftarrows} CH_2OPO_3H_2.CHOH.COO \sim PO_3H_2 + czH_2$

This reaction is suppressed by iodoacetate because the dehydrogenase is poisoned. Zymohexase (reaction (iv.)) is not appreciably affected by this poison. Accordingly when yeast treated with sodium iodoacetate acts on hexose diphosphate, owing to the differential inhibition of enzymes (p. 215) in the zymase complex, triosephosphates accumulate (see above).

It is believed that during this oxidoreduction there is a redistribution of energy in the molecule. The transferred energy becomes incorporated in the high potential phosphate bond which is combined in the carboxylic group of the acid (*i.e.*, as acyl phosphate). Evidently for reaction (vii.) to go on indefinitely dihydrocozymase must be continuously re-oxidized. In living cells, especially under aerobic conditions, there are several ways in which this may occur (p. 38 *et seq.*). When zymase cleavage is taking place *in vitro* the major method is that described below in reaction (xiii.).

(*d*) *The production of* 3-*phosphoglyceric acid by the transfer of an energy-rich phosphate bond from* 1 : 3-*diphosphoglyceric acid to ADP, and the storage of this bond in ATP.* This diphosphoglyceric acid is unstable in aqueous solution, and with ADP as specific phosphate acceptor (apparently adenylic acid will not serve), it is at a rapid rate partially dephosphorylated. No enzyme is required. Embden, extending Nilsson's and Lohmann's earlier work, had by 1933 proved that phosphoglyceric acid accumulates when yeast treated with

sodium fluoride acts on hexose diphosphate. The reason for this will be given after describing reaction (x.) below. As it is known that in the absence of fluoride, phosphoglyceric acid can be fermented, we see that its status as an intermediate metabolite is good. It can be produced and consumed during zymase cleavage.

(viii.) $CH_2OPO_3H_2.CHOH.COO \sim PO_3H_2 + ADP \rightleftarrows CH_2OPO_3H_2.CHOH.COOH + ATP.$

It should be observed that as a result of reactions (vi.), (vii.), and (viii.), an inorganic phosphate bond has become one of the energy-rich phosphate bonds in ATP, which is available as a phosphate donator for further transphosphorylations, such as those summarized in equations (i.) and (iii.).

(*e*) *The production of* 2-*phosphoglyceric acid by the intramolecular transphosphorylation of* 3-*phosphoglyceric acid, and its conversion by dehydration into* (*enol*)-*phosphopyruvic acid.* There occurs at this stage, under the agency of *phosphoglyceromutase*, another intramolecular transphosphorylation, by which 2-phosphoglyceric acid is produced. To separate this acid Meyerhof and Kiessling in 1935 exploited the fact that its barium salt is more soluble than that of the 3-phosphoglyceric acid.

(ix.) $CH_2OPO_3H_2.CHOH.COOH \overset{\text{phosphoglyceromutase}}{\rightleftarrows} CH_2OH.CHOPO_3H_2.COOH$

Enolase then causes the dehydration of the 2-phosphoglyceric acid to yield (enol)-phosphopyruvic acid, which was isolated in 1934 by Lohmann and Meyerhof as the crystalline silver barium salt.

(x.) $CH_2OH.CHOPO_3H_2.COOH \overset{\text{enolase}}{\rightleftarrows} CH_2 = CO \sim PO_3H_2.COOH + H_2O.$

During the course of reaction (x.) the energy mobilized by the dehydration passes to the phosphate bond and causes the free energy value to be raised from the 3,000 cals. of the ester linkage to the 11,000 cals. of the enol-phosphate linkage. It should be noted that (ix.) and (x.) are reversible like all the other reactions so far described except (i.). Meyerhof and Kiessling reported that in a system containing phosphoglyceric acid as the initial substrate, and phosphoglyceromutase and enolase without other interfering enzymes, equilibrium was attained when there were present 58·5 per cent. 3-phosphoglyceric acid, 12·5 per cent. 2-phosphoglyceric acid, and 29 per cent. (enol)-phosphopyruvic acid.

Fluoride poisons enolase, and so blocks reaction (x.), without appreciably influencing the cleavage of hexosephosphates (reactions (iv.) and (v.)) or the oxidoreduction centring upon phosphoglyceric aldehyde (reactions (vi.), (vii.), and (viii.)). Owing to this differen-

tial inhibition (p. 215), phosphoglyceric acid accumulates when yeast acts on hexosediphosphate in the presence of sodium fluoride. If we measure fermentation by carbon dioxide and ethyl alcohol production, we may state that fluoride greatly retards or even suppresses the process ; but we note that several of the stages in glycolysis may go on.

(*f*) *The production of pyruvic acid by the transfer of an energy-rich phosphate bond from (enol)-phosphopyruvic acid to ADP or adenylic acid.* (Enol)-phosphopyruvic acid is enzymatically dephosphorylated in the presence of either ADP or adenylic acid as phosphate acceptors. A further energy-rich phosphate bond is thus stored. It is known that magnesium ions are required, and there is a report that Warburg and Christian have, in 1942 in Germany, stated their belief that the enzyme actuating this transphosphorylation is a *magnesium protein complex.* Meyerhof has, in 1942 in the U.S.A., expressed his opinion that the reaction is truly reversible.

(xi.) $CH_2{=}CO{\sim}PO_3H_2.COOH + ADP \rightleftarrows CH_3.CO.COOH + ATP$

This reaction is poisoned by fluorides, possibly owing to the production of a magnesium fluorphosphate.

Before the work we have described was carried out it was known that pyruvic acid can be consumed and produced by yeast. The discovery that carbon dioxide is rapidly evolved when yeast cells act on α-keto acids led to the suggestion that pyruvic acid is an intermediate metabolite in fermentation. Strong supporting evidence came later (see reaction (xii.)). Then, in 1921, von Grab introduced β-naphthylamine as a fixing agent (see p. 215) into a fermenting mixture, and from 180 gm. of cane sugar got a yield of 7·3 gm. of α-methyl-β-naphthocinchoninic acid, which is a condensation product of β-naphthylamine and pyruvic acid. It cannot be too strongly emphasized that pyruvic acid is a key substance in carbohydrate metabolism, and that, aerobically or anaerobically, the steps in its formation from sugars are probably represented by equations (i.) to (xi.). Its possible aerobic fate in the higher plants will be considered later (pp. 305–6). Here we note that when alcoholic fermentation is in progress under the agency either of living cells (*e.g.*, yeast, or cells of many higher plants metabolizing in the absence of oxygen) or of the whole zymase system *in vitro*, it is decomposed in the manner we shall now describe.

(*g*) *The decarboxylation of pyruvic acid with the production of acetaldehyde and carbon dioxide.* Neuberg's first report on this reaction appeared in 1911, and led to his discovery that cells, or preparations from cells (*e.g.*, zymin), capable of fermenting sugars contain an enzyme, *carboxylase*, which can cleave pyruvic acid with the production of acetaldehyde and carbon dioxide. In a quantita-

tive experiment eighty per cent. of the theoretical amount of acetaldehyde was obtained.

(xii.) $$CH_3.CO.COOH \overset{\text{carboxylase}}{\rightleftarrows} CH_3CHO + CO_2.$$

It is by this reaction that all the carbon dioxide set free during alcoholic fermentation is derived. It is now known that cocarboxylase (thiamin diphosphate) is essential for it to occur, and that it is stimulated by magnesium ions. Phosphatases present in zymase preparations may dephosphorylate cocarboxylase, and thereby inactivate it. The addition of thiamin (vitamin B_1) sometimes leads to the reconstitution of the coenzyme. Using radioactive carbon as a labelled atom, Carson obtained some evidence in 1941 that reaction (xii.) is reversible; but the rate of synthesis of pyruvic acid from acetaldehyde and carbon dioxide is probably too slow to be of physiological significance. We note that in modern opinion it is held that pyruvic acid may be converted into hexose-6-phosphate by the reversal of the glycolytic processes represented in the equations (xi.) to (ii.) inclusive. It is important to determine what substances are converted into carbohydrates by living cells. We may regard pyruvic acid as a "probable" and acetaldehyde as a "possible."

(*h*) *The reduction of acetaldehyde to ethyl alcohol, with dihydrocozymase from reaction* (*viii.*) *as hydrogen donator.* It has long been known that traces of acetaldehyde accumulate during fermentation. Its production was, for a period, attributed to the oxidation of ethyl alcohol, but the discovery of carboxylase made it probable that it is a precursor of ethyl alcohol. By his researches on fermentation in yeast, and in the higher plants under anaerobic conditions, Neuberg (see p. 215) obtained convincing evidence that it is the immediate precursor of ethyl alcohol, which is derived from it by reduction. It is now known that this reduction is effected by the transfer of hydrogen from dihydrocozymase, formed in reaction (viii.), and occurs under the agency of *alcohol dehydrase* (see p. 38).

(xiii.) $$CH_3.CHO + czH_2 \overset{\text{alcohol dehydrogenase}}{\rightleftarrows} C_2H_5OH + cz.$$

Cozymase thus acts as a carrier between a reducer (triosephosphate) in reaction (viii.) and an oxidizer (acetaldehyde) in reaction (xiii.) in two independent enzyme systems, in which the apoenzymes are respectively triosephosphate apodehydrase and alcohol apodehydrase. These two enzymes have been obtained as crystalline proteins. We note that in zymase cleavage hydrogen transfer takes place in two oxidoreductions and phosphate transfer in several reactions. Each form of transfer plays an essential part, occurring independently, and having its own acceptor-donator system. Provided that all these systems work together effectively, as they invariably do in healthy yeast cells, and often do, but less effectively, in enzyme preparations

containing the whole zymase complex, fermentation will proceed until all the hexose substrate has been consumed with the production of ethyl alcohol and carbon dioxide as end-products. Intermediate metabolites (*e.g.*, hexosephosphates, acetaldehyde) may accumulate if they escape into the continuous phase from the surfaces of the dispersed enzymes, or if the rate of their production is faster than that of their consumption. Moreover, if in accordance with equations (i.) to (xiii.), we modify Gay-Lussac's equation (p. 49) and construct a new overall equation we see that we may regard two active phosphate bonds and two molecules of water as end-products of zymase cleavage.

$$C_6H_{12}O_6 + 2H_3PO_4 + 2\ \text{Adenylic acid (or 2 ADP)} \xrightarrow{\text{holozymase}} 2C_2H_5OH + 2CO_2 + 2\text{ADP (or 2ATP)} + 2H_2O.$$

F. Phosphorylases and their Linkage with Zymase

In 1937 Parnas described by the term "phosphorolysis" the process by which glycogen is esterified in animal cells to produce a glucose monophosphate. Cori, Cori, and Colowick, in the U.S.A., proved that this ester is glucopyranose-1-phosphate (see p. 52) and isolated from liver an enzyme, *phosphorylase*, which catalyzes *in vitro*, in the presence of adenylic acid, the cleavage of glycogen by producing phosphorylated molecules of glucose.

$$\text{Glycogen} + n\,H_3PO_4 \underset{\text{phosphorylase}}{\rightleftarrows} n\,CHOPO_3H_2.(CHOH)_3.CH.CH_2OH.$$
$$\qquad\qquad\qquad

1-phosphate (Cori ester) and glucose-6-phosphate (Robison ester), each with a phosphate bond of low energy value, is obtained.

$$CHOPO_3H_2.(CHOH)_3.CH.CH_2OH \overset{\text{phosphoglucomutase}}{\rightleftarrows} CHOH.(CHOH)_3.CH.CH_2OPO_3H_2. \qquad 6$$

Inasmuch as such a mixture at *p*H 7 and 25° Centigrade contains ninety-four per cent. of Robison ester and only six per cent. of Cori ester, it is not surprising that the Cori ester was discovered after the Robison ester. Besides, the Cori ester tends to be decomposed by hydrolysis in laboratory manipulations. We have already pointed out (p. 54) that the Robison ester is the first product of the action of holozymase on glucose. It clearly follows that *phosphoglucomutase* links *phosphorylase* with *zymase* in yeast or in other cells where glycogen is the substrate for carbohydrate metabolism (see fig. 2). For example, in the slow autofermentation that goes on in yeast cells suspended in water, it is probable that glycogen is converted by phosphorylase and phosphoglucomutase into glucose-6-phosphate, and that the reactions (ii.) to (xiii.) inclusive, which were described in Section E, then take place in succession.

In this country, Hanes (*115*) has prepared, first from ungerminated peas and later from potato tubers, a phosphorylase, which had the highly desirable property of being free from amylase and phosphoglucomutase. He showed that in aqueous solution this enzyme, in the presence of inorganic phosphate, phosphorylates starch or any carbohydrate of the starch $\rightleftarrows$ maltose series with the formation of glucose-1-phosphate (Cori ester). In the absence of amylase, starch is degraded along a single path, and the Cori ester accumulates when there is no phosphoglucomutase present to consume it. In a very successful experiment Hanes prepared in this way 79 gm. of a crystalline potassium salt, which had the composition

$$C_6H_{11}O_5.OPO_3K_2.2H_2O.$$

He gained an even more striking success when he used his phosphorylase preparation to synthesize *in vitro* a substance which had properties closely resembling those of one or more of the components of natural starch (see p. 433). These enzymic syntheses of glycogen and starch, polysaccharides possessing giant molecules, are among the major achievements of experimental biochemistry.

Hanes caused higher-plant phosphorylase to react with a relatively high concentration of glucose-1-phosphate at *p*H values of about 6. It should be noted that no adenylic acid had to be added for the reaction to occur (*cf.* the synthesis of glycogen). After a lag period,

inorganic phosphate was liberated, and samples of the solution began to give weakly the starch iodide test. Later the solution became turbid, and finally, by mechanical aggregation of the enzymically produced dissolved starch, there were deposited spherical grains, ranging in diameter from 4–10μ, and staining blue-black with iodine. Possibly the most widely known of all plant products, and one used by man since the days of ancient Egypt, was thus synthesized for the first time in history. Hanes showed that this synthesis was autocatalytic, because the lag period decreased when he added small amounts of soluble starch to the initial mixture.

Hanes prepared over 50 gm. of synthetic starch. He found that it was hydrolyzed nearly to completion by β-malt-amylase, from which he inferred that it bore a closer relation to the amyloamylose fraction of starch than to normal whole starch. Other properties (*e.g.*, the intensity of the blue colour given with iodine) were also similar to those of this fraction. He points out, however, that although synthetic and natural starches have, under X-ray examination by Astbury *et al.*, revealed essentially the same patterns, Haworth *et al.* have reported that the synthetic starch is made up of 80 to 90 glucose units, whereas natural starch is built up from 24 to 30 units. Evidently, and not surprisingly, in the separation of phosphorylase from the plastids there has been some derangement in the enzymic mechanisms which control synthesis. The surprising thing is that it is so slight. Hanes has good reasons for stating that "further investigation in this field will elucidate the range of processes, chemical and physical, which are involved in the building up from simple substances of the complex matter which forms biological structure."

Hanes regards as strictly reversible the phosphorylative and dephosphorylative reactions that he has so closely studied in his beautiful researches.

$$(C_6H_{10}O_5)_n + nH_3PO_4 \overset{\text{phosphorylase}}{\rightleftarrows} nC_6H_{11}O_5.OPO_3H_2.$$

Presumably this reaction may occur in any cell capable of producing and breaking down starch. It is not yet known what determines whether phosphorylase or an amylase/maltase system will control the mobilization of starch reserves. Here we must note that if phosphorylative degradation occurs *in vivo*, glucose-1-phosphate will not accumulate, but will be converted into glucose-6-phosphate by phosphoglucomutase. This enzyme has been found with phosphorylase in the cells of higher plants. Evidently the phosphorylase of all amyliferous plants, like that of yeast, may link with the zymase complex. For example, in the higher plants, starch may give rise to pyruvic acid aerobically by the linked action of phosphorylase, phosphoglucomutase, and the enzymes controlling reactions (ii.) to

(xi.) described in section E. It must not be overlooked, however, that some of the pyruvic acid might be formed by these same reactions with glucose-6-phosphate produced by reaction (i.) as substrate for reaction (ii.). The glucose part of the Robison ester could be derived from the amylase/maltase hydrolysis of starch or from some other source (*e.g.*, cane sugar). James has demonstrated pyruvic acid accumulation in a starch-free barley juice.

Inasmuch as reactions (xi.) to (ii.) inclusive and those controlled by phosphoglucomutase and phosphorylase are believed to be strictly reversible, we may deduce the hypothesis that any substance (*e.g.*, fat, vegetable acid, amino acid), which may in green plants be converted into pyruvic acid, serves as a potential substrate for sugar or starch production. It may be predicted that the implications of this hypothesis will be widely investigated in the future.

G. Enzymes as Thermolabile, Colloidal, Biochemical Catalysts, showing Specificity

The enzymes that have been separated from living cells appear to fulfil the principal requirements of a catalyst, in that without themselves serving as the sources of the end-products, they either accelerate reactions which can proceed spontaneously but slowly, or promote reactions which could not occur in their absence. An enzyme may enter into chemical combination during the course of a reaction, but the end-products are entirely derived from the substrate. Indeed, for reactions of short duration, at moderate temperatures and constant and favourable *p*H, with a limited amount of substrate, the concentration of enzyme will be the same at the end as at the beginning of the reaction, *i.e.*, the initial catalytic powers of the enzyme system remain unimpaired. Hence a small amount of enzyme may possess and show prodigious powers. Thus it has been calculated that one " molecule " (see footnote on p. 65) of purified catalase can decompose a hundred thousand molecules of hydrogen peroxide per second ; that one " molecule " of yellow enzyme may react twenty thousand times per minute ; and that in the presence of adequate amounts of substrate and cozymase 0·001 mg. of alcohol dehydrogenase may bring about oxygen uptake at the rate of 0·35 cubic mm. per minute.

It must be remembered, however, that enzymes are readily inactivated under unfavourable conditions, such as sometimes arise during the course of a reaction. For example, trypsin is only active in alkaline solution, and is gradually inactivated by the hydrogen ions set free when this enzyme cleaves proteins into amino acids in unbuffered solutions. This particular inactivation is reversible, for tryptic activity is restored by once more making the solution alkaline.

The velocity of an enzyme action, like that of any other catalysis,

is proportional to the concentration of the substrate, and to that of the enzyme. The quantitative relations, which depend on these concentrations, temperature, *p*H, and often on other factors, are various and complex, and, for most enzyme actions, our knowledge has, until recently, been obscure. They have formed subjects for much discussion in special monographs (*e.g.*, Bayliss, *19*; Haldane, *111*) and articles (*e.g.*, Moelwyn-Hughes, *204*) on enzymes. More satisfactory evidence concerning the kinetics of certain enzyme actions has been obtained in recent experiments in which purified enzymes and coenzymes have been used under strictly controlled conditions. Thus Dixon (*78*) proved that dismutation of acetaldehyde proceeds stoichiometrically in accordance with the equations given on p. 49; that whereas the initial velocity for a given substrate concentration varies with the enzyme concentration, the reaction proceeds to completion at all enzyme concentrations; that the initial velocity for a given enzyme concentration varies with the initial substrate concentration; that the most rapid rate of change occurs at *p*H 7·7; and that the enzyme is thermolabile.

Experiments have shown that, if the reaction catalyzed by an enzyme is reversible, the enzyme, like an ordinary catalyst, will accelerate both reactions to about the same extent. Consequently, the *position* of equilibrium will not be appreciably altered. It is important to note, however, that the *rate* at which equilibrium is reached may be greatly accelerated. The metabolic significance of these findings is discussed elsewhere (p. 28).

With few exceptions enzyme preparations do not dialyze, and estimations of their molecular weights [1] by physical methods (*e.g.*, determinations of the rate of diffusion or of sedimentation in an ultracentrifuge) have given numbers (*e.g.*, 36,000 for pepsin, 260,000 for catalase) of the same order as the molecular weights assigned to certain proteins, polysaccharides, and other giant molecules. Enzymes, therefore, have always been regarded as colloidal catalysts, which develop extensive active surfaces when dispersed in a reaction medium. It is supposed that enzymic reactions take place on these surfaces. Certain enzymes (*e.g.*, lipase) can actually bring about chemical change when dispersed in liquids (*e.g.*, water, or ethyl alcohol, for lipase) in which they are quite insoluble. Such enzymic reactions resemble surface catalyses induced by certain metals (*e.g.*, colloidal platinum). As might be expected, the degree of dispersion of enzymes appears to exercise an important influence on surface activity. Evidence exists that dispersion is affected by temperature, *p*H, and other environmental factors.

But it can hardly be denied that chemical forces also govern and

[1] Evidently this term is used loosely, since it has not yet been definitely proved that enzymes are single chemical compounds.

direct enzyme actions. The whole surface of an enzymic micella or molecule may be chemically active, or there may be active areas on the surface. The chemically active regions would possess affinities for and powers of effecting change in its appropriate substrate or substrates. What may happen during the reaction is that the enzyme combines chemically with and activates the substrate, which is then decomposed. The products of the reaction may have little affinity for the enzyme. Accordingly by diffusing from the enzyme surface, they would make room for further molecules of substrate.

Enzymic activity may sometimes be reduced or stopped either by narcotics or by traces of inorganic poisons. It is supposed that substances (*e.g.*, phenylurethane) included in the former class of inhibitors are adsorbed by enzymes, and so occupy the active surfaces to the exclusion of the substrate molecules. Certain inorganic poisons (*e.g.*, mercuric chloride) may act by precipitating the dispersed enzyme. Others may combine chemically at the active centres, as happens, for example, when oxidation enzymes containing iron are inactivated by hydrogen cyanide or hydrogen sulphide.

It has long been known that most enzymes, when dispersed in water at temperatures greater than about 50° C., are gradually and irreversibly inactivated, *i.e.*, they are thermolabile. The higher the temperature the more rapid is the inactivation. The enzymic powers of aqueous systems can usually be completely destroyed by boiling for a few minutes. When coagulation of protein accompanies inactivation there is evidently an enormous decrease of surface. Moreover, spontaneous chemical changes may destroy active centres. It should be noted that temperature has another effect on enzyme action, viz., that of accelerating the chemical changes involved. Such an accelerating effect occurs in all chemical reactions, whether catalyzed or uncatalyzed. The value of the temperature coefficient (see p. 234) is usually about 2. Up to a certain temperature, this effect predominates in a given enzyme action, under defined conditions. Above that temperature, the inactivation of the enzyme by heat becomes relatively more powerful and consequently the whole process is retarded. The temperature at which these two opposing effects of heat balance has been described as the optimum temperature. This, however, is not a constant, even for a given enzyme preparation. It varies according to the conditions of the experiment, the time factor (see p. 283) playing an important part. In experimental work enzyme actions are usually hastened by allowing them to progress at temperatures between 30° C. and 40° C.

More recently it has been established that the *p*H of the medium may have far-reaching effects upon enzyme activity. An unfavourable *p*H may destroy enzymic properties: *e.g.*, yeast invertase undergoes fairly rapid inactivation at *p*H values less than 3.

Optimum values of *p*H have been determined for certain enzyme preparations under defined conditions, and lie, for certain plant enzymes, between 4 and 5, a range not infrequently met with in plant cells. Doubtless one effect of *p*H is upon the dispersion of ampholytic components (*e.g.*, proteins, see p. 449) of enzyme preparations, but electrical effects concerned with the mode of ionization of ampholytes or the adsorption of hydrogen or hydroxyl ions may also play a part. It is possible that some form of chemical change occurs at active centres, when enzymes are irreversibly inactivated in solutions which are too acid or too alkaline.

The term specificity of enzymes implies that the chemical structure of substrates exerts an influence on enzymic catalyses. There is no universal enzyme which can act on all metabolites, and Nature has not been so lavish as to produce a specific enzyme for every metabolite. Simplifying what is an exceedingly complex analytical study (see Haldane, *loc. cit.*, chap. VI), one may state that individual enzymes have been separated which appear to be specific either for a single compound (but this is rare), or for a single type of chemical linkage which may occur in many different compounds. Catalase may be cited as an enzyme which appears to be specific for a single compound, viz., hydrogen peroxide. Usually, however, an individual enzyme can act on more than one compound (see notes on enzymes, section C). Linkages which are attacked by a given enzyme may be sparsely distributed among metabolites, or the name of possible metabolites may be legion. All proteins are hydrolyzed by the proteases, all starches by the amylases, and all fats, and indeed other substances possessing the ester linkage, by the lipases. In contrast, invertase can act on but few substrates because its activity is specific for a linkage, viz., that between glucose and fructose in cane sugar, that is not widely distributed in natural products. This linkage is found, however, in certain trisaccharides ; consequently, these compounds are hydrolyzed by invertase. Thus raffinose yields fructose and melibiose (p. 26). The hydrolysis of raffinose into cane sugar and galactose by melibiase provides further evidence of specificity. Evidently the linkage between galactose and glucose in the melibiose molecule is attacked. The specificity of the prunase component of emulsin is towards substances containing a β-glucoside linkage (*e.g.*, prunasin, sambunigrin, salicin, raffinose), while maltase acts only on α-glucosides. Specificity among the oxidases may either relate to the oxidizable substances or to the oxidizing agent. Catechol oxidase in the presence of oxygen appears to act only on substances that contain a catechol grouping. *In vitro*, hydrogen peroxide appears to be the proper substrate for peroxidase. Flavoproteins and dihydrocodehydrases are reaction partners.

The specificity of an enzyme complex (*e.g.*, zymase, p. 50) should

be distinguished from that of the simple enzymes and coenzymes of which it may be compounded. For instance, zymase in the absence of cophosphorylase is specific for certain hexosephosphates, but in the presence of cophosphorylase, phosphates, and magnesium salts, the whole zymase complex is assembled, and specificity becomes extended to *d*-glucose, *d*-fructose, and *d*-mannose. The structural relationship of these hexoses is indicated by the fact that they have a common enolic form (p. 430). It is noteworthy that *d*-galactose, which would give a different enol, is not fermented. Apozymase itself is complex: among other active components, it contains zymohexase which is specific for hexosephosphates; a dehydrase, which in the presence of cozymase acts on triosephosphate, and another which in the presence of dihydrocozymase acts on acetaldehyde; and carboxylase, requiring cocarboxylase, which is specific for certain α-ketonic acids (*e.g.*, pyruvic acid).

H. The Ordered Metabolism of Whole Cells, and Autolysis

Complex enzyme systems, such as zymase, may be regarded as fragments of protoplasm which have escaped disintegration during the processes of extraction and purification. Certain complex systems for the existence of which there is good evidence, *e.g.*, the cytochrome oxidation system, dehydrase-cytochrome-oxidase (p. 35), have not yet been separated as wholes. It may be that a more complex fragment consisting of the whole cytochrome system, flavoprotein, together with zymase, would effect the complete aerobic oxidation of sugar, *in vitro*. Continuing with this conception of synthesis from active fragments, we arrive at the conclusion that the whole protoplasm is compounded of thermolabile complex systems and single enzymes, and of associated physiologically active substances (*e.g.*, hydrogen ions, activators, inhibitors, etc.). Thus zymin contains zymase, invertase, emulsin, protease, peroxidase, dehydrases, etc., *i.e.*, a single cell may accommodate many different enzymes. The extensive enzymic surfaces in the colloidal protoplasm of any given cell will, owing to their specificity, direct metabolism along certain lines: for example, glucose is fermented by yeast, yielding alcohol and carbon dioxide, but is cleaved to lactic acid by myozymase in the muscle cells of animals. Anabolic as well as catabolic events are governed by these surfaces, *i.e.*, enzymic micellæ govern specific syntheses as well as specific degradations. Accordingly, under favourable conditions *in vivo*, syntheses by enzymes such as esterases, phosphorylases, amidases, amino acid oxidases, and emulsin, may be more efficient than *in vitro*, and enzymic syntheses that could not be foreshadowed by studying the reactions of single enzymes *in vitro*, may govern and direct growth processes. It is evident that during growth the balance between

anabolism and catabolism is in favour of the former. Now anabolism requires energy. Apart from the absorption of solar energy by green cells, it is the energy released in catabolic processes, in particular in respiratory oxidations, that is used (p. 260). Hence it may be inferred that in the whole protoplasm enzyme systems may be coupled to each other in such a manner as to enable centres of synthesis to obtain and use the energy liberated elsewhere. If this is granted, it follows that it will be found impossible to carry out certain biochemical syntheses *in vitro* until such time as the proper coupled systems are assembled under suitable conditions. The words in the last two sentences were written in 1935. New knowledge about the activities of amidases now provides us with good illustrative examples of the idea they express. Asparaginase and glutaminase act hydrolytically *in vitro*. Syntheses of the amides asparagine and glutamine have been achieved *in vivo* (see Chibnall, *59*) when certain plant tissues were fed with ammonium aspartate or ammonium glutamate, but not as yet *in vitro*. They are endothermic changes. Krebs claims to have shown that synthesis by animal tissue of glutamine from ammonium glutamate is dependent upon a supply of respiratory energy. Chibnall considers the assumption reasonable that respiratory energy promotes the synthesis by rye grass leaves (p. 204) of glutamine from ammonium glutamate. Relating to this idea is the fact (p. 53) that the energy-rich phosphate bonds in ADP and ATP may effect phosphorylations that do not take place under the agency of less energy-rich phosphate bonds, such as occur in ester phosphates. Evidently the cophosphorylases play an important part in coupled metabolic reactions. It is noteworthy that some of these reactions have been achieved *in vitro*. In their important article Wohl and James (*335*) discuss further examples of intermolecular and intramolecular energy transfers.

Finally, we note that ordered metabolism in a cell is governed not only by the specificity of enzymes, and the coupling of anabolic and catabolic processes, but also by the physical structure of protoplasm, which may regulate the migration of substrate molecules, and, consequently, the order and rate of enzymic changes. Injury to cell-structure leads to disordered metabolism, which is often called autolytic metabolism or autolysis, and end-products not normally present in healthy cells may then accumulate. Thus, owing to the hydrolysis of prulaurasin by prunase, hydrogen cyanide is produced by cherry laurel leaves autolyzing in chloroform vapour. Furthermore, glycosides may be hydrolyzed under natural conditions as cells age and die. For instance, coumarin glucosides yield *o*-coumarin in the dying cells of harvested sweet vernal grass and of the shoots of sweet woodruff in the late spring. In apples, pears, and certain other fruits, at a late stage of storage, or after mechanical or frost injury,

disordered metabolism may lead to the accumulation of considerable amounts of ethyl alcohol and small amounts of acetaldehyde. It has been suggested that such spontaneous or induced alterations in the physical structure of protoplasm disturb the normal relations between zymase and other enzymic systems, and occasion unregulated zymasis. Very striking colour changes often accompany autolysis. A reasonable explanation is that the oxidation systems (direct oxidase, tyrosinase, etc.), substrates (polyphenols, tyrosine, etc.), and oxygen which, when brought together, yield brown, red, blue or black oxidation products, do not commingle in healthy cells, and that obstacles to the free diffusion of suitable substrates and dissolved oxygen are removed by injury to the physical structure of the protoplasm. In fine, a comparison of normal metabolism with autolysis, compels belief in the controlling influence that the physical organization of protoplasm exerts on the total chemical behaviour of living cells.

PART II

THE ABSORPTION, TRANSLOCATION, AND ELIMINATION OF WATER, SOLUTES, AND GASES

THERE are several reasons why a plant cannot grow without water. First, and above all, turgor is a necessary condition for growth. Again, water is essential for plant life because it is the solvent in which metabolites migrate, the medium for metabolic change, and is itself a reacting component in certain important metabolic events (*e.g.*, photosynthesis and hydrolyses).

The water for land plants is supplied by the soil, which also provides essential elements for plant nutrition in the form of mineral salts. The water and solutes are absorbed by the root system and are conducted to all parts of the plant. Across parenchyma water passes by osmosis, and solutes pass by diffusion. In the vessels and tracheides of the xylem there is a mass movement of solutions. Some of the water is retained, but much is lost by transpiration. The mineral salts, together with water and carbon dioxide, are used in the manufacture of metabolic products. Some of these dissolve in water, and the conduction of organic solutes, either by diffusion (*e.g.*, across parenchyma, and according to Mason and Maskell in sieve tubes) or by passive carriage in moving sap (*e.g.*, in the lumina of vessels and tracheides, and, according to Münsch, in sieve tubes), is an essential operation in plant nutrition. Loss of solutes by the external secretion of solutions is rare and of little quantitative significance, but solutes are lost when leaves, etc., fall, or when bark is shed. Possibly woody perennials thus get rid of toxic waste products. In association with the processes of photosynthesis and respiration, gases pass in and out through stomata and lenticels, and diffuse in the intercellular spaces.

CHAPTER IV

THE VACUOLATED CELL AS AN OSMOTIC SYSTEM [1]

PLANT cells cannot absorb solids. They are dependent for their vital activities on a continuous supply of nutritive liquid and of water-soluble gases. The properties of non-living cell walls and of protoplasmic membranes govern the passage of water, and determine which solute molecules can enter or leave living plant cells. In a first analysis the osmosis of water and the diffusion across membranes of solutes may be regarded as independent processes. Instances are known of the rapid passage of water with the slow diffusion of solutes, and, under conditions favourable for the absorption of solutes, of the accumulation in all parts of a plant of certain solutes (*e.g.*, potassium salts) at rates that are not evidently related to those of the absorption and conduction of water.

A. The Classical View of the Water Relations of a Vacuolated Cell [2]

The osmotic pressure of cell sap. A vacuolated living plant cell may be regarded as an osmotic system. Like a parchment membrane the cellulose cell wall imbibes water, and the wet wall readily allows the passage of water and solute molecules in crystalloidal solution. But for our discussions in this section the permeability of the fully imbibed nucleated protoplast that lines this wall in vacuolated cells is, for simplicity, compared with that of a porous pot impregnated with copper ferrocyanide, *i.e.*, we shall consider that a healthy protoplast possesses the properties of a truly semipermeable membrane. Actually many vacuolar substances can pass in and out through protoplasts, but, as a rule, only at a slow rate (see section C). Since, by comparison, water passes rapidly, our assumption that protoplasm is a semipermeable membrane often approaches the truth.

The cell sap of plants consists of many solutes (*e.g.*, molecules of glucose, fructose, and cane sugar, and molecules and ions of mineral salts, and of organic acids and their salts) dissolved in water. Some are dissolved in colloid and others in crystalloid solutions. The osmotic pressure of cell sap is governed by the number of particles present in unit volume, and is independent of their nature. Its magnitude varies greatly from plant to plant, from tissue to tissue in

[1] This chapter should be read in conjunction with Appendix II, sections D and E.

[2] Non-vacuolated cells (*e.g.*, meristematic tissue) of plants imbibe water and swell, in a manner analogous to the swelling of gelatin (p. 456).

a given plant, and from cell to cell in a given tissue. Apparently it is rarely less than 3·5 atmospheres, even in starved cells. In storage organs rich in sugar, it may be greater than 20 atmospheres, and a value of 40 atmospheres (approximately) has been found in the fruit of the grape. Largely owing to the high concentration of sodium chloride in the sap, the cells of halophytes have exceedingly high osmotic pressures, sometimes over 150 atmospheres (see also p. 77).

Plasmolysis and recovery from plasmolysis to a state of turgor. When isotonic solutions of different substances are placed inside and outside a rigid semipermeable membrane (see fig. 3), endosmosis and exosmosis of water will occur at equal rates, and the level of the liquids will not change. With a hypotonic solution outside, endosmosis of water takes place, making the solution outside more concentrated, and diluting the solution inside. The osmotic pressure of the external solution continues to increase, and that of the internal solution to diminish until the solutions become isotonic. With a hypertonic solution outside, equilibrium is reached by the exosmosis of water until the solutions become isotonic.

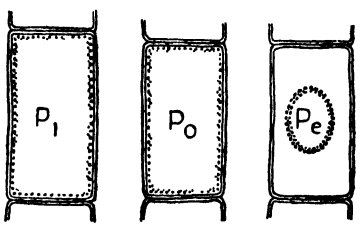


FIG. 3. Changes in the osmotic pressure of cell sap during plasmolysis (see text).

If a turgid vacuolated cell containing sap that has an osmotic pressure P_1 is immersed in a hypertonic solution of cane sugar of known osmotic pressure P_e, cell sap and bathing solution can become isotonic by the exosmosis of water, and in this way only if the protoplast is semipermeable. Cell membranes differ from rigid pots in being elastic, and exosmosis at first causes the *whole* cell to shrink. Let us suppose that the volume of the whole cell thus diminishes from an initial value V_1 to a minimum value V_0,[1] and that the osmotic pressure of the sap increases to P_0. At this minimum volume the protoplast is just pressing against the cell wall over the whole surface of the latter, *i.e.*, the cell just possesses turgor. Further exosmosis occurs, however, provided that the bathing solution is still hypertonic towards the cell sap (*i.e.*, $P_e > P_0$); and, owing to the resulting diminution in the hydrostatic pressure of the vacuolar solution, the protoplast, which is highly elastic, will withdraw from the cell wall.

[1] The amount of contraction shown will be determined by the initial volume (V_1) of the cell and by the mechanical properties of the cell wall. If we start with a fully turgid cell (p. 76) having a volume V, and if at limiting plasmolysis it has a volume V_0, the quotient V/V_0 represents what is called the degree of turgor stretching. Owing to differences in the properties of cell walls the quotient for cells from comparable tissues of different plants may vary widely. This fact probably possesses ecological significance (see p. 124).

Plasmolysis has occurred ; the cell has lost its turgor, and there is a space between protoplast and wall that is filled with the bathing solution (see fig. 5).

Plasmolysis is readily demonstrated with cells containing coloured plastids, since the pigments make the shrinking protoplasts easy to see. And plasmolysis is readily observed in red and blue tissues, since healthy protoplasm is impermeable to anthocyanins ; it should be noticed that the colour intensifies owing to the diminution in the volume of the cell sap. Plasmolysis continues until the cell sap becomes isotonic with the bathing solution. If, as is usual, the volume of the bathing solution greatly exceeds that of the tissue, we may neglect the volume of water that has passed out from the cells during plasmolysis, and state that equilibrium is reached when the osmotic pressure of the cell sap has increased to P_e, *i.e.*, we may regard the osmotic pressure of the bathing solution as being only imperceptibly reduced by the trifling dilution which results from exosmosis.

If the protoplast has not been injured during plasmolysis, and thus may be regarded as still possessing the properties of a semipermeable membrane, the plasmolyzed cell will recover from plasmolysis when it is transferred to pure water. Plasmolysis in a hypertonic solution followed by deplasmolysis in water indicates that a cell is in a healthy state. This recovery is a result of the endosmosis of water. The vacuolar sap increases in volume, and, when the osmotic pressure of the cell sap has fallen to P_0, the cell will have regained turgor.

Methods of determining the osmotic pressure (P) of cell sap. (*a*) Surround a vessel containing the tissue with a mixture of ice and salt. Allow the frozen tissue to thaw, and then express the sap with a hand press. Determine the freezing point ($-\triangle$ ° C.) of the expressed sap. The average osmotic pressure of the sap of the tissue in atmospheres is approximately equal to $12\triangle$. For more accurate determinations, the molar strength of a solution of cane sugar freezing at $-\triangle$ ° C. should be found. The osmotic pressure of the sap will be the same as that of this solution of cane sugar. To ascertain its value tables giving the osmotic pressure of cane sugar over a wide range of concentrations should be consulted.

(*b*) Measure the volume of the cell (V), and place the cell in a hypertonic solution of cane sugar of known osmotic pressure (P_0), The whole cell will first contract, and then plasmolysis will take place and proceed until the osmotic pressure of the sap has increased to P_0, owing to the contraction of the solution in the vacuole to a volume V_0. This should be measured. Then $P = P_0V_0/V$.

Model experiments for method (*b*) have been performed by Bennet-Clark *et al.* (*25*). They placed, at 20° C., thin sections (*e.g.*, of two varieties of beet, B and C) in solutions of cane sugar of graded

osmotic pressures, and injected the intercellular spaces by a method similar in principle to that described on p. 154, so bringing the plasmolyzing solution (the plasmolyticum) into close contact with the cells. After half an hour they counted the percentage number of cells showing plasmolysis in each plasmolyticum. This period was selected because they found that in slightly hypertonic solutions the operation of plasmolysis was not completed after fifteen minutes, but the percentage of plasmolyzed cells was not increased by prolonging the immersion beyond thirty minutes. For every tissue examined they obtained sigmoid curves when they plotted in charts (fig. 4) the percentage degree of plasmolysis against osmotic pressure. The range in osmotic pressures which may be shown by cells in a single tissue is strikingly wide. Some cells in beet B had osmotic pressures as low as 9·5 atmospheres, the maximum being 16 atmospheres. It is also noteworthy that a different range, viz., from

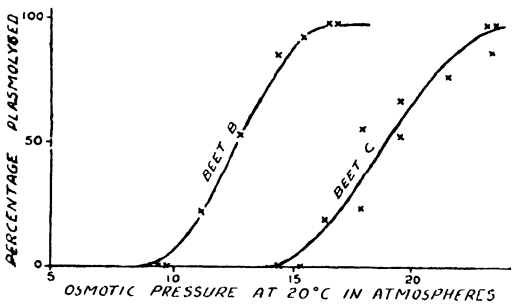


FIG. 4. From Bennet-Clark, Greenwood and Barker, *25* (see text).

about 14 to 25 atmospheres, was shown by a second variety, beet C. From these charts the osmotic pressure of that sugar solution in which fifty per cent. of the cells in any tissue are plasmolyzed can be found by dropping a perpendicular from the fifty value on any curve to the x-axis. This value was always at the point of inflection of the sigmoid curve. It clearly follows that the corresponding value read off on the x-axis is that of the osmotic pressure of an *average cell* when it is in the state of "limiting plasmolysis," *i.e.*, when plasmolysis is just visible. For beet B this value was 12·6 atmospheres and for beet C 18·4 atmospheres. Bennet-Clark and his co-workers have thus "by careful and accurate counting" determined what we have called above P_0, and they rightly point out that many heretofore have been content with "a rough visual impression obtained by a cursory examination of the tissue."

The suction pressure of turgid cells. After turgor has been regained in a cell recovering from plasmolysis (p. 74), turgor pressure (T), *i.e.*, the hydrostatic pressure exerted by the cell sap against the

protoplast and the cell wall, increases upon the further endosmosis of water. This entry of water will be opposed, however, by the inwardly directed pressure of the cell wall. This pressure, which is always equal but opposite to the turgor pressure, will, evidently, become progressively greater as the cell enlarges. (*Cf.* the progressive increase in the resistance offered by a bicycle tyre to the pumping of air into an inner tube.)

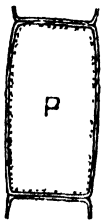


FIG. 5. The same cell as that illustrated in Fig. 3 when fully turgid.

The term suction pressure (S) denotes the net pressure that causes water to enter a plant cell. For an isolated cell in pure water, the *full suction pressure* is at the outset exerted, and, if the imbibitional capacities of cell wall and protoplasm are ignored, is equal to the osmotic pressure (P) of the cell sap (*i.e.*, the force causing water to enter) less the wall pressure (*i.e.*, the force opposing entry).

$$S = P - T \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (i.)$$

Clearly, during deplasmolysis, T is zero and S is always equal to P, and decreases as P decreases. When turgor has been regained, wall pressure enters as a factor; and as the cell volume increases, the

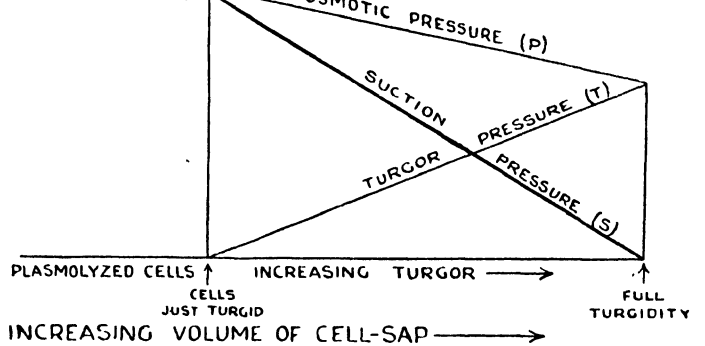


FIG. 6. Changes in the magnitudes of osmotic pressure, suction pressure, and turgor pressure, accompanying changes in volume of cell sap. (From Thoday, *300*, modified.)

decrease in P is accompanied by an increase in T, *i.e.*, the suction pressure progressively diminishes (fig. 6). Finally, a state is reached when S becomes zero, because T = P. The cell has attained its maximum volume (V); it is fully turgid (see fig. 5).

For a cell taken from a land plant, V is usually markedly greater than the original volume (V_1) of the cell when removed from the

plant. Under natural conditions tissues of land plants are rarely fully turgid, and expand when placed in water: *e.g.*, the diameters of circular discs of carrot root increase, and the hydrostatic pressures that are developed often fracture the discs. It should be noticed that only at full turgidity does the hydrostatic pressure of the cell sap (*i.e.*, turgor pressure) become equal to the osmotic pressure of the sap. Thus when we stated (p. 73) that the osmotic pressure of the sap of halophytes sometimes exceeds 150 atmospheres, we did not imply that the hydrostatic pressure in the cells even approached this high value. These plants live in salt water, and would have to be transferred to pure water for full turgor to be developed. Actually the cells would be killed by the high pressures developed, long before they became fully turgid.

For cells in media other than pure water, forces other than wall pressure may oppose the entry of water. For instance, when a plant cell is placed in a hypotonic solution possessing an osmotic pressure P_e,

$$S = (P - T) - P_e \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad \text{(ii.)}$$

and, clearly, for water to enter it is necessary that $P - T$ should be greater than P_e, *i.e.*, in a cell possessing moderate turgor, the osmotic pressure of the cell sap must be considerably higher than that of the bathing solution. A fully turgid cell (*i.e.*, a cell that has been immersed for a period in pure water) shrinks when it is placed in a bathing solution hypotonic with respect to its cell sap.

Equation (ii.) suggests methods by which the *full suction pressure* (see equation (i.)) of a cell or a tissue may be readily determined. The experimental material is immersed in different solutions of cane sugar of known molar strength, and hence of known osmotic pressure. The full suction pressure of the cells may be taken as being equal to the osmotic pressure (P) of that solution in which the weight or the volume of the material remains unchanged: for, clearly, in this solution, S is zero, and, consequently, $P - T = P_e$. Stiles and Jørgensen showed that tissue from a potato tuber neither gained nor lost weight in M/4 cane sugar, and concluded that the full suction pressure of this potato was 6·5 atmospheres. For tissue from carrot root, the full suction pressure worked out at 17 atmospheres. The volume method may be illustrated by experiments with short strips cut from the inflorescence stalk of the dandelion. The average full suction pressure of the tissue parenchyma is equal to the osmotic pressure of a solution of cane sugar in which no change of curvature, *i.e.*, of volume of the parenchyma, occurs (see p. 471). The fact that we are dealing with the *full suction pressure* requires emphasis. This is the *initial pressure* with which water is absorbed when the tissue is placed in pure water. The suction pressure steadily diminishes as the tissue increases in turgor.

B. Some Facts Unexplained on the Classical View

Bennet-Clark *et al.* (*25*) use the term *water-absorption pressure* (A_l) to denote the osmotic value of cell sap as determined by their plasmolytic method (p. 75) when cells are in a state of limiting plasmolysis and have, on the average, a volume of V_l. For this state the terms water-absorption pressure and full suction pressure, as defined above, seem to mean the same thing. These investigators determined A_l plasmolytically for a given tissue, and expressed the juice from the same tissue, when the cells were in their natural state and had an average volume of V_t. The osmotic pressure (P_t) of this expressed juice was determined cryoscopically. It follows that the osmotic pressure of this juice would have been P_tV_t/V_l had the average cell volume been that (V_l) shown at limiting plasmolysis. The argument then ran that according to the classical view the value of ($A_l - P_tV_t/V_l$) should be zero, since this view states that, when cells lack turgor, water absorption depends solely upon the magnitude of the osmotic pressure of the vacuolar sap. Within the limits of experimental error it was zero for some tissues, but for others positive values of from 2·2 to 7·1 atmospheres were obtained. It was thus discovered that for a given cell state the water-absorbing power may be considerably in excess of the average osmotic pressure of the cell sap. The nature and origin of this excess pressure of x atmospheres had thus become defined by Bennet-Clark's researches as a fundamental problem of plant physiology, and it is engaging his active attention (Bennet-Clark and Bexon, *24*). Here we note that he has now decided not in future to describe the extra process as the secretion of water into the vacuole. He takes into account such knowledge as we possess of anomalous diffusion in physical systems, and visualizes in living tissue a dynamic system dependent upon the diffusion of solute molecules and ions which, in their movements, may carry with them shells of water. He considers that the system is under metabolic control (see also p. 18), the nature of which he is striving to elucidate.

In the experiments of Bennet-Clark *et al.* the cells in which $A_l = P_l + x$, and, accordingly, in which metabolism may influence water movement, belonged to potentially growing tissues (*e.g.*, beetroot, swede). We shall point out later (p. 90) that the absorption of solutes by tissues capable of growth may also be under metabolic control. In the experiments on rhubarb and Caladium x was zero and $A_l = P_l$, *i.e.*, the water relations were explicable on the classical view. This was confirmed when it was shown that fifty per cent. of the cells in a tissue became plasmolyzed when a section from it was mounted in juice expressed from the remainder of the tissue. In contrast, when x was strongly positive, no cells were plasmolyzed when they were mounted in their own juice. In all of the cells, even those with the lowest value of A_l, the water-absorption pressures exceeded the average osmotic pressure of the vacuolar saps, and exosmosis of water, if it occurred, was not sufficient to cause plasmolysis.

By means of experiments of a very different kind Bennet-Clark and Bexon (*23*) obtained supporting evidence for their objections to the classical theory as a complete explanation of cell-water relations. The validity of these objections is still a matter for debate.

C. The Solute Relations of Vacuolated Cells

The necessary conditions for the penetration of solutes into living cells. Although protoplasm generally allows the passage of water

more readily than that of dissolved solutes, the growth of plants at the expense of materials contained in the environment, and the presence of solutes in the cell sap, force one to admit that protoplasm is not a truly semipermeable membrane, and that many solutes can penetrate through cell walls and protoplasm into vacuoles.

Elsewhere (p. 465) we point out that in physical systems with crystalloid solutions inside permeable membranes and pure water outside, endosmosis of water is always accompanied by exodiffusion of solute. Hence it appears that the movements of solute and solvent are controlled by different causal factors. Nevertheless the diffusion of a solute, by altering the osmotic pressure of solutions on two sides of a membrane, may bring about secondary movements of water.

We have seen that positive suction pressures occasion the passage of water into cells, and in the present section we shall consider, among other matters, the factors that govern the absorption of solutes (viz., the permeability of cell membranes, the steepness of the diffusion gradients from source to sink, and the fate of the diffusing solute at the sink).

The permeability of cellulose cell walls. It is supposed that cellulose cell walls, like collodion or parchment membranes (p. 452), readily permit the passage of substances in crystalloid solution but do not allow that of solutes in colloid solution. When cell walls become lignified, cutinized, or suberized, or when they become associated with hemicelluloses or mucilage, their permeability alters. Some non-living dermal coverings actually become semipermeable, *e.g.*, the pericarp of barley grains is semipermeable towards dilute sulphuric acid.

Plasmolysis experiments confirm the view that inorganic salts, sugars, and other solutes can pass through cellulose cell walls; for the solutes must reach the outer surface of the shrinking protoplast to bring about the exosmosis of water from the cell sap. Also it can be shown that the exodiffusion of soluble vacuolar pigments, sugars, and other substances, is not stopped by cell walls when the protoplasm of plant cells (*e.g.*, cells of red beet) is injured by raising the temperature above 40° C., by treating the cells with chloroform, or by immersing the cells in decinormal acid or alkali. So far as we know none of these conditions, which are injurious to protoplasm, affects the normal properties of cell walls.

The experimental investigation of the permeability of protoplasmic membranes. We cannot by studying the behaviour of physical models obtain even presumptive evidence concerning the permeability of the protoplasmic membranes. Every living tissue has distinctive properties, which may be modified by changes either in the internal or in the environmental conditions. Consequently the gathering of experimental data under defined conditions is the first requirement in

this complex study. The following notes are based on the valuable survey of Stiles (*258*).

(*a*) *Chemical and physical investigations on expressed sap and on external liquids.* One can determine what substances have been absorbed from an external liquid of known composition by performing chemical tests upon expressed sap of the plant under experiment. For example, the sap expressed from washed mustard seedlings, which have been grown in a solution of potassium nitrate, turns blue when treated with diphenylamine in the presence of strong sulphuric acid. This is a colour test for nitrates. Hence we conclude that the seedlings have absorbed nitrate ions from the external liquid. As a control experiment the sap expressed from seedlings that have been grown in water should be subjected to the same treatment. As a rule no blue colour is given.

Much information has been gained about the absorption and excretion of solute molecules by performing, over a period, a series of quantitative tests on the liquid that surrounds a plant tissue. Thus we may find out by chemical analysis whether a given solute is absorbed, and, if it is, we may then follow the course of absorption. Or, if the solute is an electrolyte, conductivity changes may be measured. For acidic and alkaline substances, changes in hydrion concentration may be measured electrometrically, or by means of suitable indicators. In all three types of experiment it must be remembered that endodiffusion may be accompanied by exodiffusion. The extent of exodiffusion may be estimated by immersing comparable samples of the tissue in pure water and determining the changes in chemical composition, electrical conductivity, or hydrion concentration, that occur in the external liquid over the same period as that used in the experiments on absorption.

(*b*) *Visible or easily detected changes in cells.* We cannot conclude from the results of experiments of the type described in (*a*) that protoplasts have been penetrated by the solutes that are absorbed or excreted, for, conceivably, interchange might have taken place between the cell wall and the external solution. For certain solute particles, penetration into the vacuolar sap is readily demonstrated with the aid of a microscope. Thus we may observe that, first, an intense blue colour appears, and then a blue precipitate gradually accumulates in the vacuoles of Spirogyra or in the mesophyll cells of many green leaves when filaments of the alga or sections of the leaves are kept in dilute aqueous solutions of methylene blue. By direct observation the permeability of plant cells towards other coloured solutes has been tested, and informative results have been obtained.

Several types of visible or readily detected change may result from the penetration of non-pigmented solute particles. Thus crystals of calcium oxalate are produced when calcium ions reach the vacuoles of the cells in the roots of *Dianthus barbatus*, because the sap in these cells contains free oxalic acid. Caffeine penetrates rapidly into the vacuoles of Spirogyra and combines with phenolic substances to yield a grey precipitate. Changes in the colour of the petals of certain flowers may be brought about by injecting the intercellular spaces with weakly acidic or weakly alkaline solutions. Starch formation in certain leaves, which have been floated in the dark on solutions of sugars and other organic solutes (p. 217), demonstrates that these solutes reach the plastids of the cells.

(*c*) *Plasmolysis experiments.* If a cell when immersed in a hyptertonic bathing solution of a single substance becomes plasmolyzed, one may infer that the protoplast is more permeable to water than to the dissolved solute.

It is not necessary that the protoplast should be quite impermeable to the solute. Plasmolysis might, indeed, be brought about by a strongly hypertonic solution of a solute to which the protoplasm is readily permeable, provided the living membrane were relatively more permeable to water. Two very important facts should be carefully noted about plasmolysis that is effected by solute molecules to which protoplasm is permeable, viz.: (i.) a higher concentration of solution is necessary to cause plasmolysis than would be required were the protoplasm impermeable to the solute; and (ii.) a cell that has been plasmolyzed in a solution of a solute to which it is permeable may recover from plasmolysis after prolonged immersion in this solution. Use has been made of these two facts to obtain numbers that indicate the relative permeability of protoplasm towards substances in solution.

(i.) PERMEABILITY COEFFICIENTS. Since cane sugar penetrates protoplasm extremely slowly, solutions of this compound have been taken as standards in attempts to measure the permeability of protoplasm to other compounds. Experiments are performed to determine the molar strengths of solutions of different compounds that bring about the same degree of plasmolysis in the cells under investigation as is effected by a solution of cane sugar of known molarity, due regard being paid to the effects of ionization when electrolytes are used.

Let us suppose that an x molar solution of cane sugar just causes plasmolysis. Then, were the protoplasm impermeable to another non-electrolyte, this other compound should also in x molar solution just bring about plasmolysis. Should, however, the protoplasm be relatively more permeable to this other compound, a greater molar strength would be required, *i.e.*, the isotonic coefficient as determined by this plasmolytic method would be less than that found by a physical method (p. 471). Thus in a class experiment it was shown that the cells in Spirogyra filaments were plasmolyzed in 0·2 M cane-sugar, but remained turgid in 0·5 M ethyl alcohol. Plasmolysis was observed in M ethyl alcohol. The cells after treatment with 0·5 M ethyl alcohol could be plasmolyzed in strongly hypertonic cane sugar and deplasmolyzed in water, *i.e.*, the protoplasts had not been injured.

For an electrolyte, not an equimolar but a physically isotonic solution would have to be used in the first place. Were the protoplasm impermeable to the electrolyte, this isotonic solution would also cause plasmolysis, but were the protoplast permeable, a stronger solution would be required. Again, the isotonic coefficient obtained by physical methods would differ from that determined by plasmolytic methods.

Numbers for the relative permeability of a given protoplast to different solutes have been obtained from the relation $\mu = 1 - i'/i$, μ being the coefficient of permeability in respect to the solute under investigation, i the isotonic coefficient as determined by a physical method, and i' the isotonic coefficient as calculated from the results of plasmolysis experiments. For a solute in a solution to which the protoplast is truly semipermeable, $i = i'$, and therefore μ is zero. For a solute to which the protoplast is permeable, i' will be less than i, and μ will work out at some positive value between zero and unity. The fraction i'/i will diminish as the protoplast shows increasing permeability to individual solutes in a set of solutions containing different solutes, *i.e.*, μ will approach unity.

(ii.) PLASMOLYSIS FOLLOWED BY DEPLASMOLYSIS. When the protoplast of a cell undergoing plasmolysis in a hypertonic solution of a single salt has reached maximum contraction, the osmotic pressure of the cell sap will be equal to that of the solution which occupies the space between the cell wall

and the protoplast. The concentration of the solute in this solution will, however, still be higher than its concentration in the vacuole ; for in the vacuole this solute is only one component of a mixture of substances, each one of which contributes to the osmotic pressure of the sap. Hence, if the protoplast is not truly semipermeable, this solute will diffuse into the vacuole. The osmotic pressure of the cell sap will thus be increased, and, when the external solution becomes hypotonic towards the cell sap, water will pass in from the external solution, and the volume of the sap in the vacuole will increase, *i.e.*, the cell will begin to recover from plasmolysis.

It should be noted that the failure of a cell to recover from plasmolysis does not necessarily mean that its protoplast is impermeable to the solute in the solution which induced plasmolysis. For example, exodiffusion of solute particles from the vacuole might compensate for the endodiffusion of this solute from the bathing solution. Or, as happens when cells of the red beet are plasmolyzed in solutions of sodium chloride, the solute in the bathing solution passes into the vacuole, but the osmotic pressure of the cell sap does not increase because the diffusing solute is either adsorbed or forms an additive chemical compound, *i.e.*, the total number of particles in the vacuole remains unchanged.

Filaments of Spirogyra have frequently been used for experiments of this kind. Thus de Vries, who was the first to point out the significance of recovery from plasmolysis, noticed that cells in a sample of *Spirogyra nitida* were strongly plasmolyzed in less than half an hour by 3·3 per cent. glycerol, and that they had become turgid again after twenty-four hours. In 6·9 per cent. glycerol the protoplast first separated into ellipsoidal and spheroidal masses, but after two days many of the cells were again turgid. He concluded that after two days the osmotic pressure of the cell sap of the deplasmolyzed cells had increased to that of a 6·9 per cent. solution of glycerol, since the deplasmolyzed cells remained turgid when the filaments were transferred to solutions of potassium nitrate, sodium chloride, glucose, and cane sugar, isotonic with 6·9 per cent. glycerol. Numbers purporting to represent the permeability of protoplasm to various solutes have been obtained by measuring the rate of recovery from plasmolysis. Usually the cell under investigation is first plasmolyzed in a cane sugar solution of known molar strength (say, C gm. mols per cc). The volume (V_1 ccs) of the vacuolar sap enclosed by the contracted protoplast is then calculated from measurements made with a standardized micrometer eye-piece. After this has been done, the cell is transferred to a solution containing the solute whose power of penetrating the protoplast is to be gauged. This solution should be isotonic (as judged by physical methods) with the cane sugar solution. Clearly, if the protoplast is as little permeable to this solute as it is to cane sugar the volume of the vacuolar sap will not alter. But for a solute that can penetrate protoplasm, a gradual increase in volume of the vacuolar sap will be observed. Let us suppose that the increase is to V_2 after t minutes. Then the amount of solute that has passed through the protoplast in one minute will be $(V_2 - V_1)C/t$ gram molecules. The whole outer surface of the protoplast is exposed to the external liquid, and by determining the mean surface area exposed by the protoplast during the experiment, one may express permeability to the solute as gram molecules penetrating per unit area per unit time. By this method Lepeschkin calculated that (from 67 to 183) $\times 10^{-9}$ gm. mols of glycerol passed through each square centimetre of protoplast surface of Spirogyra cells per hour. We note that permeability towards a given solute may vary from cell to cell.

The permeability of a membrane may be defined as the amount of solute

passing in unit time through unit area of the membrane when there is unit difference between the concentrations of the diffusing solute in the solutions on opposite sides of the membrane. Criticisms of plasmolytic and of other methods purporting to give numbers expressing permeability have been made, because the importance of taking into account the magnitude of the concentration gradient has been ignored.

(*d*) *Electrical conductivity as a measure of the permeability of protoplasts to ions.* Healthy plant tissues strongly resist the passage of electrical currents, but the resistance decreases when the protoplasts are injured. In other words, the electrical conductivity of healthy tissues is low, but increases when protoplasm is injured. Since conductivity in liquid systems depends upon the migration of ions, Osterhout supposed that dissolved ions can only migrate slowly across healthy protoplasts, and in certain important researches (p. 85) used change of electrical conductivity (which is a measure of the rate of migration of ions) as a measure of change in the permeability of the protoplasts in the constituent cells of living tissue to the migrating ions.

General conclusions arrived at from experiments on the permeability of protoplasts.[1] Although protoplasts are usually more permeable to water than to solute molecules, it appears that most substances that dissolve to give crystalloid solutions can penetrate protoplasmic membranes. For a given tissue the rate of penetration depends upon the nature of the solute and upon the environmental conditions. Further, the permeability of the cells of a given tissue to a given solute may change as the tissue ages. We note also that cells of different tissues under identical environmental conditions may show very different permeabilities to a given solute.

(i.) *Permeability and the nature of the solute.* It is usual to find that healthy protoplasts of plant cells are only slowly penetrated by sugars, amino acids, and inorganic salts, *i.e.*, substances of importance in nutrition. Protoplasm appears to be more rapidly penetrated by glycerol, glycol, and other polyhydric alcohols, and it is extremely permeable to ethyl alcohol. There is also good evidence that compounds containing certain nitrogenous groups (*e.g.*, urea, urethanes, purines and other substances containing amide or imide groups) may sometimes enter cells rapidly. Facts such as these may throw light on the composition and structure of plasmatic or other bounding membranes. One may, for the present, picture these membranes as thin films composed of protein and lipoid material. The protein part absorbs water and permits the passage of inorganic salts, sugars, and other solutes (*e.g.*, certain dyes) that are insoluble in lipoids but soluble in water. Moreover, substances (*e.g.*, other dyes) that dissolve in lipoids are readily absorbed by plant cells. It is supposed that they pass in through the non-aqueous (*i.e.*, lipoid) phase of the plasmatic membrane.

[1] Critical discussion of the subject matter of this and later sections of this chapter will be found in the books of Stiles (*288, 291*) and Gray (*99*).

Dyes have been much used in experiments on permeability because it is usually easy to see whether they have penetrated into living cells, and how far. Only those dyes that dissolve to give crystalloid solutions can pass into the vacuoles of plant cells. The cell wall prevents the passage of colloid solutions of dyes. Moreover, it is far easier to observe the penetration of basic dyes (*e.g.*, methylene blue), since these may accumulate in the vacuole owing to combination with phenolic and other acidic substances in the sap. It is sometimes difficult to decide whether acidic dyes have penetrated, for the colour of the cell sap when equilibrium is attained may be very faint.

(ii.) *Permeability changes occasioned by environmental agencies, and by metabolic products.* Changes in permeability to water as well as to solute particles may be brought about by varying the external conditions; and many investigations have been made on the effects produced by variations of light intensity, temperature, and of the hydrion concentration of the external liquid. The effects of single inorganic salts and pairs of salts, and of narcotic and toxic substances, have also been much studied.

Such alterations in conditions as may lead to injury cause permeability to increase. Irreversible increase is followed by the death of the cell, which often undergoes post-mortem change of colour (in the apple, for example, brown oxidation products are produced). Tissue of the red beet provides suitable material for studying the conditions that lead to an increase in permeability. The protoplast of a healthy cell is impermeable to the anthocyanin pigment dissolved in the cell sap, but, upon injury, this red pigment diffuses out of the cell. Experiments show that exodiffusion occurs fairly rapidly from washed discs placed in chloroform water, decinormal hydrochloric acid, decinormal sodium hydroxide,[1] or in hypotonic solutions of sodium chloride, at ordinary temperatures; and in water at temperatures greater than 40° C. Moreover, the pigment passes out rapidly at ordinary temperatures from discs which have been frozen and thawed rapidly. Thus it appears that the relatively low permeability of healthy protoplasts is only maintained within certain limits of temperature and hydrion concentration, in solutions containing more than one inorganic salt (see also p. 85), and in the absence of toxic substances.

Toxic metabolic products sometimes accumulate in ageing plant cells, and may cause an irreversible increase in permeability. Such increase leads to the injury and death of cells. It has been suggested

[1] The anthocyanin of beet loses its colour in alkaline solution, but flavonic substances pass out also, and turn yellow in the external liquid. Flavonic substances do not pass out through the protoplasts of healthy cells that are placed in neutral water.

that some of the so-called physiological diseases of stored fruits are thus caused by acetaldehyde and other toxic metabolic products. Thus the flesh tissue of certain varieties of apple and pear suffers injury when the fruit is stored at temperatures less than 5° C. There is little doubt that changes that lead to an irreversible increase in permeability play an important part in the sequence of events that leads to tissue browning. Clearly, fundamental researches, such as those of Stiles (*292*) upon the effects of toxic substances and of injurious conditions on the permeability of protoplasm, may have important practical applications.

Reversible changes in permeability, which may play an important part in governing the behaviour of certain plant organs, are sometimes termed functional changes in permeability. Such changes may be brought about as direct responses to alterations in environmental conditions. Thus there is evidence that diurnal and seasonal fluctuations in permeability may be effected by diurnal and seasonal fluctuations of temperature and light intensity. Reversible changes in permeability may also be controlled by metabolic events, such as photosynthesis and respiration. Touching these functions one may note that importance has been attached to the hydrion concentration of cell sap. In photosynthesis, carbonic acid is changed into a neutral substance; and, in respiration, carbonic acid and, possibly, other acidic substances are produced. Much depends upon the buffer capacity of cell sap; but it has been suggested that diurnal alterations of hydrion concentration may sometimes occur (*e.g.*, in stomatal guard cells), and by affecting the state of cell proteins induce functional changes in permeability.

(iii.) *Antagonism.* The effects of solutions of single and of pairs of inorganic salts on permeability are still imperfectly understood, but certain curious facts seem to be well established. It appears that the permeability of protoplasts increases when cells are immersed in solutions of a single salt of sodium, potassium, or magnesium. Prolonged immersion leads to injury. The permeability of the protoplasts often decreases at first when cells are placedi in solution containing a single salt of calcium, but over a longer perod the effect of calcium ions in the absence of other metallic ions is the same as that of sodium, potassium and magnesium ions, namely, to increase the permeability and to injure the cells.

Many years ago, Osterhout made the remarkable discovery that this effect of the metallic ion in a single salt in bringing about an increase in permeability may be antagonized by adding to the solution a relatively small amount of a second metallic salt containing a different metallic ion. Very striking antagonism was displayed between salts of monovalent and divalent metals in Osterhout's experiments on the electrical conductivity of the thallus of Laminaria

in different saline solutions. The electrical conductivity was low in sea water, but progressively increased after the thallus was transferred to an isotonic solution of sodium chloride, *i.e.*, the permeability of the cells to ions progressively increased. In isotonic calcium chloride there was first a decrease and then an increase in electrical conductivity. When the thallus was transferred from sea water to an isotonic solution of a mixture of sodium chloride and calcium chloride in which the concentration of the sodium salt was in considerable excess over that of the calcium salt, there was hardly any alteration in the electrical conductivity. The calcium ions, even in low concentration, had effectively antagonized the injurious influence of the sodium ions.

Phenomena of antagonism may be encountered when cells of the higher plants are placed in solutions containing two or more solutes. For example, Bayliss showed (*cf.* p. 84) that the permeability of the cells of red beet to anthocyanins was increased by transferring discs of tissue from pure water to hypotonic sodium chloride, but that less exodiffusion of anthocyanin occurred when the transfer was made from water to a hypotonic solution containing a small amount of calcium chloride in addition to sodium chloride.

As a result of the extension of Osterhout's pioneer work it is now generally agreed that there must be a suitable balance among the various ions (anions as well as cations) that are dissolved in solutions outside plant cells, in order to maintain the relatively low permeability possessed by healthy cells. Sea water, the fresh water of pools and rivers, and soil solution, may be regarded as being suitably balanced for the cells that these liquids bathe. Much experimental work has been carried out with a view to determining the proportions in which nutrient salts should be used in order to provide a well-balanced culture solution for the growth of plants.

The position of equilibrium attained when diffusible solutes are absorbed by plant cells. (i.) *Differences between the composition of cell sap and external solution.* When two different crystalloid solutions are separated from one another by means of a permeable membrane, the solute particles, whether molecules or ions, tend to diffuse until their concentrations become the same throughout the system (p. 465). This tendency must always exist where there are permeable membranes and concentration gradients. If there is a heterogeneous system inside the membrane, as happens in living cells and in certain physical models in some ways analogous to living cells, the final distribution of diffusible particles, especially of ions, may be profoundly affected. Adsorption may take place on internal surfaces and if colloidal ions are present in the heterogeneous system (and they are always present in cytoplasm), tendencies towards the occurrence of Donnan equilibria (p. 465) would be expected. Both

of these contributory causes may result in differences at equilibrium between the concentrations of diffusible solutes inside and outside the permeable membrane, whether living or non-living. Recent work suggests, however, that in their relations to diffusible solutes living cells may differ from any model so far constructed in that their metabolic activity may exercise a predominant influence on the rate of absorption of solutes and on the final amounts absorbed (see pp. 90, 108), and render difficult to detect the purely physical influences, which must also be at work. One of the major recent physiological researches has been the investigation of the absorption of inorganic solutes by living cells.

Comparisons of the composition of plant sap with that of a solution outside the plant definitely indicate that it is rare for a diffusible substance to exist at the same concentrations inside and outside living cells (see Stiles, *288*). Thus the figures given in table II show that the concentration of certain ions in the cell sap of the large marine alga Valonia differ from those of the same ions in sea water. This coenocytic alga was selected for experiment, since several cubic centimetres of sap can be expressed from the large central vacuole. Accordingly accurate quantitative analysis of sap can be performed. Similar results have been obtained with Nitella (see p. 90).

TABLE II. *Differences between the ionic composition of the cell sap of Valonia and sea water*

	Parts per 1,000	
	Sea water	Cell sap
Cl'	19·6	21·2
$Na\cdot$	10·9	2·1
$K\cdot$	0·46	20·14
$Ca\cdot\cdot$	0·45	0·07
$Mg\cdot\cdot$	1·31	Trace
SO_4''	3·33	0·005

Only general statements can be made for land plants. The salt composition of the sap expressed from a *whole* plant usually differs widely from that of the solution in which it has been grown. Variations occur, however, from tissue to tissue in a given plant, and in a given tissue at different stages of development. Also we must remember that different plants growing together may make different demands upon the mineral substances in the soil solution. Until much more is known about the behaviour of single cells and of

isolated tissues in solutions of known composition, it is useless to face questions so difficult as those presented when one considers *whole* land plants in relation to diffusible solute particles.

(ii.) *The preferential absorption of ions.* Experiments have disclosed the surprising fact that living cells generally absorb cations and anions from a solution of a single salt at different rates. (*Cf.* a physical system consisting of a colloid solution of protein inside a parchment membrane, and a crystalloid solution outside (p. 465).) Thus Patanelli and Sella discovered that the roots of *Cucurbita pepo* preferentially absorbed anions from solutions of chlorides, sulphates, and phosphates, of potassium and calcium. And Redfern found that, after thirty-six hours, roots of *Pisum sativum* had absorbed far more calcium ions than chlorine ions from a solution of calcium chloride. In decinormal solution, 17·7 per cent. of calcium ions and 3·6 per cent. of chlorine ions were simultaneously absorbed. Smaller differences were found with more dilute solutions.

When preferential absorption occurs, electrical equilibrium is maintained in various ways. Ionic exchange is one of them. In the experiment described above Redfern noticed that potassium and magnesium diffused out of pea roots while the excess of positively charged particles was passing in from the solution of calcium chloride. Hoagland (*134*) states that the preferential absorption of nitrate ion from a solution of calcium nitrate may be in part balanced by the diffusion into the external solution of the bicarbonate ions that are produced from the carbon dioxide set free in respiration. Some physiologists have held that if, as appears to be probable, there is a connection between the absorption of solutes and respiration, it should be attributed exclusively to this ionic exchange (see p. 90). The electrical neutrality inside and outside a cell, when ions of a dissolved solute are being absorbed at different rates, may also be maintained by the passage in or out of an excess either of the hydrion or the hydroxyl ions contributed to the system by the dissociation of the solvent, water. For example, when the cation M is preferentially absorbed from a salt MX dissolved in water, as often happens in water culture experiments, electrical neutrality might be maintained by the passage in of hydroxyl ions in electrical equivalence to the excess of positive ions absorbed from the solute. Accordingly the acidity of the external solution would increase. Such an increase has been observed during the course of water-culture experiments.

(iii.) *The course of absorption and the absorption ratio for single salts.* Stiles and Kidd by measuring the change in the electrical conductivity of solutions of single salts placed outside cut discs of certain storage tissues were able to follow the course of the absorption of these salts. By plotting the amount of solute absorbed against time, they obtained a logarithmic curve. This indicated that the further away the

system was from equilibrium the more rapid was the rate of absorption. Plainly it is not the permeability of cell membranes which alone determines the rate of penetration of a dissolved solute. Account must also be taken of the equilibrium conditions for the dissolved solute in the cell under experiment. For example, it is conceivable that experiments might fail to show the absorption of a substance to which the cell membranes are permeable, were this substance already present in the cell and equilibrium conditions by chance satisfied when the cell is immersed in a solution of the substance.

Stiles and Kidd also determined the ratio of the final internal concentration i to the final external concentration e. This they termed the absorption ratio i/e. In a typical experiment with sodium chloride and carrot tissue they found that the absorption ratio varied with the initial concentration, and was unity for only one of the initial concentrations. They showed that it is a property of the living cell to maintain different concentrations of the salt inside and outside the cell; for, on killing the tissue, the absorption ratio approached unity. For sodium chloride solutions of normalities 0·0002, 0·002, 0·02, and 0·1, the absorption ratios worked out at 46·7, 27, 3·5, and 0·83 respectively. As would be expected from the physical laws of diffusion, with increase in the initial external concentration, the rate of absorption and the absolute amount of salt absorbed increased; but the absorption ratio (which indicates the relative amount of substance absorbed) decreased, being more than fifty times higher at the lowest than at the highest initial concentration. The absorption ratio decreased as the initial concentration was raised. With the lower concentrations the salt heaped up inside the cell, and with the higher concentrations the absorption ratio became less than unity. Stiles and Kidd pointed out that this is what would happen were salts adsorbed on protoplasmic or other surfaces after entry, and educed from their results some evidence in support of this view when they found a linear relation to hold between log i and log e (see p. 458). But they never maintained that they had proved absorption to be a process of adsorption (Stiles, *291*, p. 72). Nevertheless, Stiles (*288*) considered it a reasonable hypothesis that adsorption of electrolytes on intracellular surfaces might account for the absorption by living cells of ions against a concentration gradient.

During the course of his later experiments Stiles (*290*) made the interesting observation that conditions that favoured the aeration of solutions (*e.g.*, shaking, removing stoppers from bottles) also promoted the absorption of solutes. Using well aerated solutions in a specially designed apparatus, Steward found that the duration of absorption by discs of storage tissue could be extended very considerably, and some of the absorption ratios he obtained for dilute solutions of

potassium bromide were greater than 1,000. Moreover, he proved that the absorbed ions penetrated the protoplasts of the storage cells, and accumulated in the vacuoles. Hoagland and Davis (see below) had earlier argued from the results of their experiments on Nitella that ions may be absorbed against a concentration gradient and accumulate as free ions in the vacuoles. These experiments have brought to light new facts about the behaviour of living cells, and we note that whereas Hoagland and Davis, and Steward and his collaborators, emphasize the connection between the metabolic activity of living cells and their power to accumulate solutes in their vacuoles, other investigators (*e.g.*, Osterhout, Brooks, Briggs, Petrie) have considered and still are considering the extent to which knowledge of the behaviour of physical models, and of various kinds of Donnan equilibria, which imply ionic exchanges (see p. 88), may contribute to the elucidation of the experimental facts known about the distribution of ions between living tissues and the environment (see Stiles, *291*, Hoagland, *133*, Steward, *285*).

Since both cation and anion of a diffusible electrolyte may heap up inside a cell (*i.e.*, the absorption ratio for each ion may be greater than unity), it follows that absorption may go on after the product of the internal concentrations of the ions has become greater than the product of their residual concentrations outside the cells. No simple explanation of such results can be given in terms of Donnan equilibria (see p. 465). Briggs has pointed out, however, that conditions existing in a living cell may lead to a complicated series of Donnan equilibria. Cytoplasm is a heterogeneous system composed of many phases, in each of which there are indiffusible cations and anions (*e.g.*, protein ions), and he has suggested a number of different possibilities which might account for some of the observed distributions of ions in the environment, vacuoles, and the various phases of the cytoplasm.)

Metabolic activity as a controlling factor in the absorption of salts against concentration gradients. It is clear from the data in table II that Valonia absorbs potassium ions against a concentration gradient. Working on *Nitella clavata*, Hoagland and Davis found that the conductivity of the cell sap expressed from the multinucleate internodal cells, which are often several inches long, of this alga was twenty-five times greater than that of the pond water in which it was growing, and concluded that inorganic salts accumulated in the vacuoles and were electrolytically dissociated, and that the ions were dispersed and not adsorbed. From the results they obtained for individual ions it was evident that movement of some of the cations (*e.g.*, potassium) and anions (*e.g.*, chloride) present in the sap had taken place from lower to higher concentrations. "Mass action relations, Donnan effects, protein isoelectric points, may all be, and

probably are, of utmost importance to the functioning of the cell, and yet fall far short of explaining the final distribution of ions." The general conclusion drawn was that plant cells must perform work in absorbing ions. Careful studies on the absorption of bromide, chloride, and nitrate, by *green* cells elicited the interesting result that the accumulation of these ions was considerably augmented in the presence of light. Hoagland and Davis (see *133*, *137*, *138* and *139*) attributed this increase in absorption to the enhanced metabolism and growth that resulted from the photosynthesis of carbohydrates. Emphasis was thus laid on the intimate relationship between absorption and energy exchanges. More recently (see Hoagland, *134*), experiments have been carried out with radioactive isotopes in order to trace movements of ions into Nitella cells. Brooks found that radioactive rubidium entered "the protoplasmic phase extremely rapidly and reached a high net concentration, much higher than that of the external solution. Entry into the vacuole was, however, very slow and during the experimental periods the concentration of rubidium in the vacuole did not reach so high a value as that in the protoplasm." Broyer and Hoagland observed that radioactive bromide, after accumulating in the protoplasm, moved into the vacuole and reached a higher concentration there than in the protoplasm or in the external solution.

Steward and his collaborators (see *285* and *286* for bibliography) have obtained evidence that, provided aerobic metabolism with the liberation of respiratory energy is vigorous and provided conditions are in general suitable for growth, but only under such conditions, potassium and bromide ions may be absorbed against a concentration gradient by discs of storage tissue of the potato, artichoke, and carrot, and by plant roots, and that these ions accumulate in these tissues. They made the surprising and important discovery that the rate of respiration and of salt absorption were often simultaneously depressed upon lowering the concentration of oxygen in the environment, and, accordingly, concluded that oxygen concentration is one of the factors which may govern the rate of salt absorption (see table III). Since accumulation of cations as well as of anions was observed, and for other reasons, they rejected the possible interpretation that anion accumulation results *entirely* from the exchange of bromide for such bicarbonate ions as are produced from respiratory carbon dioxide, (*cf.* p. 88), and held that it is "the energy value of the oxidation which is the factor most probably involved."

It appears that whereas high respiration is one of the necessary factors for rapid absorption, it is not in itself a sufficient cause.

[1] Bromide was selected for use because it is not toxic, and because no difficulties arise from fluctuating values in a control experiment since this anion is absent from cell sap under natural conditions.

TABLE III. *Effects of oxygen tension upon the relative rates of absorption of K· and Br' by carrot and artichoke discs from* 0·00075 *N potassium bromide at* 23° *C., and upon the relative rates of respiration of these discs.* (*Data from Steward, Berry, and Broyer,* 286).

	CARROT			ARTICHOKE		
Oxygen, per cent.	Relative respiration	Relative absorption, K	Relative absorption Br'	Relative respiration	Relative absorption K	Relative absorption Br'
2·7	44	22	42	63	12	17
12·2	78	96	86	85	74	76
20·8	100	100	100	100	100	100
43·4	106	117	118	112	100	103

Thus in some of Steward's experiments, high rates of carbon dioxide output were shown by tissues which absorbed feebly. Apparently, what is required for high absorption is intense general metabolic activity, of which the rate of carbon dioxide output is, in some tissues, a reliable as well as a convenient measure. For storage tissues the conclusion drawn is that the principal factor in salt absorption is " their ability for renewed active metabolism and even growth, and consequently the amount of salt accumulated is closely related to the rate of aerobic respiration, which represents the major source of energy for the whole system."

The maintenance of diffusion gradients. Although peculiarities associated with the metabolic activities of certain tissues may mask purely physical effects, there is no reason to abandon as yet the straightforward view that the absorption and passage out from cells of solutes are in the first place determined by the existence of permeable membranes and of diffusion gradients. Under these conditions it is clear that the duration of diffusion will depend upon the fate of the diffusing solute. Solutes entering living cells may be variously dealt with. It has been suggested (p. 89) that they may be adsorbed on cell surfaces. Further, they may combine with a cell component, as happens when methylene blue reaches the vacuoles of cells containing tannins or simple polyphenols, or they may undergo metabolic change, as happens when starch formation follows the penetration of sugar molecules into mesophyll cells of leaves floated on solutions of various sugars.

One may suppose that a diffusion gradient will be maintained until the diffusing solute is no longer removed from the medium of diffusion. Thus methylene blue continues to enter cells containing tannin until

the external solution no longer contains the dye or until all the tannin has combined with the dye (see remarks on p. 466 concerning an analogous physical system).

Although simple chemical combination as well as adsorption may occur in growing plants under natural conditions, it is by metabolism that diffusing substances are usually removed. When a diffusing substance is consumed in an irreversible metabolic process equilibrium can never be reached. Thus equilibrium is never attained when yeast is fermenting glucose solution. During fermentation glucose continuously diffuses to the zones in the yeast's protoplasm that possess zymase activity, and is there fermented. The ethyl alcohol and carbon dioxide that are produced continuously diffuse out of the cell. Fermentation goes on until all the glucose has been consumed or until the cells have been killed by the alcohol that is produced.

For the higher plants under natural conditions diffusion gradients are maintained by the continuous replenishing of solutes at sources of supply (soil solution, air, storage cells, etc.), and the incessant metabolism in consuming cells which serve as sinks. Moreover, by cell multiplication, new sinks are created throughout the growing period, and, as we have seen, recent work suggests that young cells possess a high capacity for absorption, and can absorb salts even against a concentration gradient.

CHAPTER V

NOTES ON SOILS[1]

A. Soil Texture

THE term soil is used to denote the uppermost horizon in the profile of detritus that extends *viâ* the subsoil to the underlying igneous or sedimentary rock. Soils (other than leaf moulds and peat) owe their origin to the weathering of native rock. The process of soil formation is initiated by mechanical weathering through the agency of water, ice, and wind. This causes the comminution of mineral particles which, however, retain their chemical identity. Chemical weathering acts on some of the comminuted particles. Whereas some of the present-day soils have been derived from the weathering of the underlying rock, others have been carried, in an earlier geological period, to their present situations by river, ice, or wind. In addition, substances contained in organisms living above ground often become incorporated in the soil. Thus the soil receives deciduous members of living plants, droppings of animals, and whole organisms after death. Soils nearly entirely composed of organic matter result from the localized accumulation of plant remains. Thus a litter of leaves in deciduous forests quickly decays to form leaf mould; and, over lengthy periods, deposits of plant remains have by slow carbonization and compression yielded peats. Furthermore, we may distinguish the soil horizon from underlying horizons by the fact that it supports life. It is the milieu of a mixed society of roots, earthworms, and a most varied assemblage which is included under the terms microflora and microfauna. The substance of these organisms and of plant members living underground also becomes incorporated in the soil, after their death.

In addition to mineral matter and organic matter, all soils contain soil air and soil moisture. By the cultivator's art, mineral matter, organic matter (already present or added), and moisture, are amalgamated into crumb-like particles which, in association, give the characteristic porous structure of productive soil of good tilth. Earthworms in temperate, and termites in tropical regions, feed on soil and mix and excrete moist crumb-like particles. In this way they play an important part in the development of the structure of uncultivated soils. In all soils the alternate freezing and thawing, which occurs in the winter, is another factor of great importance in

[1] The highly complex physical and chemical problems included in Pedology, the scientific study of soil, have been discussed in monographs (Russell *255*, and Robinson, *250*), on which the present writer's notes are based.

the production of soil crumbs. In soils that are not waterlogged soil air occupies the space between these crumbs. This space is now regarded as cellular and not capillary (see Keen, *162*). The composition of the air which it contains depends on the respiratory activity of the living population, and on the porosity of the soil. Thus in grassland there may be three times as much carbon dioxide (1·5 per cent.) as in arable land (0·5 per cent.). Under conditions favourable for plant growth, soil air is saturated with water vapour.

It is convenient when considering certain of the physical properties of soils to group mineral particles according to their size. For this purpose special methods of mechanical analysis are used to separate the various fractions. Mineral particles, the shapes of which are actually highly diverse, are treated as if they were spherical, and equivalent diameters are then calculated. The International Society of Soil Science has decided to group fractions thus :—

Fractions.	Diameter limits (mm.).
Coarse sand	2·0—0·2
Fine sand	0·2—0·02
Silt	0·02—0·002
Clay	< 0·002

Many soils (*e.g.*, loams) are mixtures containing all these fractions. The properties of such soils will, of course, be intermediate between those of soils composed nearly entirely of coarse sand or of clay.

There is little cohesion between particles of coarse or fine sand, and although the surface of each particle may be wetted with water there is no imbibition. Consequently the water-holding capacity of sand masses is low, and water readily percolates through them to the water table below. On the other hand, there is never a shortage of air in sand masses ; they are always open soils.

As the diameters of the particles in the fractions decrease, surface properties become more emphasized. Thus, the cohesiveness and water-holding capacity of silt, though considerable, are much less than those of the fraction denoted as clay. Furthermore, clay possesses distinctive properties. For example, when moistened it imbibes water and swells. The sticky mass so formed is impervious to air and water (*cf.* sands), is plastic, and can be moulded into any desired shape. The object so produced shrinks on drying or baking, but remains whole if sufficient compression has been used in the moulding process. Cracks appear, however, in drying clay soils.

The particles belonging to the clay fraction are of very different size grades, as may readily be shown by shaking a clay with water. The relatively coarse particles quickly settle, but other particles do not settle at all, and will pass through a filter paper. Examination under the ultramicroscope shows that the filtrate is a colloid solution

containing dispersed particles in Brownian movement. Cataphoresis experiments prove that the dispersed particles carry a negative charge. Russell (*255*) has stated that "the properties of clay are now associated with: (1) The constitution and the very small size of its particles: (2) their affinity for water; (3) the negative electric charge which they take on in the presence of water." The clay colloid is in the gel state in soils, and, as Robinson (*250*) has stated, "is pre-eminently the reactive constituent of the mineral portion of soil."

The physical properties of sands, silts, and clays, may be greatly altered in the presence of organic matter. A distinction may be made between recognizable plant remains and animal remains, and the dark amorphous product (conveniently termed humus) which results from the decomposition of such remains. Humus is a complex colloid aggregate, and gives rise to the organic gel of soils. It provides certain essential elements of the food supply of plants. It is also highly important because of its physical properties. Thus it gives cohesive properties to and augments the water-holding capacity of sands. Together with the undecomposed plant remains it tends to make clay soils more open. Colloid clay and colloid organic matter associate to give a single colloid complex.

Special mention must be made of calcium carbonate. This mineral, though absent in very open sands and vegetable peats, is present in most soils. The amount present is high in soils formed by the weathering *in situ* of chalk or limestone. Its association with clays and silts is a matter of considerable importance. Under the influence of dissolved carbon dioxide calcium carbonate is changed to the soluble bicarbonate. The positively charged calcium ions, by neutralizing the negative charges of the dispersed particles in colloid clay, may flocculate the colloidal clay, and the aggregate of crumbs so formed becomes permeable to air and water. The soil thus acquires a good tilth. Unfortunately calcium ions are readily washed out of soil by rain, and by deflocculation colloid clay may be regenerated. In agricultural practice, lime (calcium hydroxide) is added in order to improve the tilth of heavy soils. Secondary advantages may accrue. Too great a soil acidity may be corrected, and the growth of certain important micro-organisms may thereby be promoted.

B. The Organic Matter in Soil

The dead vegetable matter that becomes incorporated in soil consists of a mixture of mineral salts, which are immediately available for plant nutrition, and of complex organic molecules, which must undergo a series of decompositions before the essential elements they contain can be re-absorbed by green plants. These residual organic compounds largely consist of cell wall substances. Substances containing nitrogen represent but a fraction of the whole mass. The

ratio of carbon to nitrogen in dead vegetable matter varies from 25/1 to 40/1. Usually traces of organic compounds containing sulphur or phosphorus are also present.

The decomposition of organic compounds is for the most part effected by saprophytic fungi and bacteria. The compounds are consumed as foodstuffs by these organisms, and some of the metabolic products are excreted into the soil. Thus the aerobic oxidation of carbon compounds leads to the production of carbon dioxide and water. The carbon dioxide, upon diffusing through the pore spaces of the soil into the air, becomes once more available for the nutrition of green plants. Catabolism of nitrogenous substances yields amino acids, amides, and ammonium salts (the fate of which will be discussed below), and that of organic compounds containing sulphur or phosphorus yields sulphates or phosphates, which may be immediately absorbed by roots.

Humus, the dark brown amorphous material of the soil, consists of partially decomposed organic matter. Owing to loss of carbon by aerobic oxidation while humification under the agency of the microflora of soil is in progress, the ratio of carbon to nitrogen diminishes from the value mentioned above to about 10/1 to 12/1. Humus is a complex mixture of organic compounds, the composition of which will, of course, vary according to the source of the humus. The results of chemical analysis suggest, however, that certain types of substance may be present in all forms of humus. Thus, on treating humus with cold alkali, certain acidic substances dissolve, while a nitrogenous residue termed humin remains undissolved. Attempts have been made to separate single acidic substances from the soluble fraction, and much discussion has centred on the chemistry of the product known as humic acid. Odén regarded this acid as free from nitrogen, and assigned to it the formula $C_{60}H_{52}O_{24}(COOH)_4$, but it is not yet generally accepted that he experimented with a single substance.

It should be noted that the dead individuals of the hypogeal microflora and microfauna also contribute to the organic matter of the soil. Of particular interest are certain groups of heterotrophic bacteria (nitrogen fixers) which possess the power of using molecular nitrogen for the synthesis of protein. Energy for the synthesis is derived from the oxidation of organic foods which these organisms absorb from humus.[1] Thus *Clostridium Pasteurianum*, an anaerobic organism, while fixing 2–3 mg. nitrogen, decomposes 1 gm. of carbohydrate; and *Azotobacter chroococcum*, an aerobic organism, can fix

[1] *Cf.* the nitrogen-fixing bacteria which live symbiotically with green plants (*e.g.*, *Bacillus radicicola* in the nodules of the roots of leguminous plants) and derive energy for protein synthesis from the carbohydrate foods made by the plant.

10 mg. nitrogen per gram of carbohydrate oxidized. Plainly, through the activity of these bacteria, soil will become richer in complex organic compounds containing nitrogen. When the bacteria die, these compounds will be acted on in the usual way by saprophytic fungi and bacteria, and by humification and hydrolysis will be converted into amides, amino acids, and ammonium salts. In rich cultivated soils (*e.g.*, those in market gardens), loss of nitrogen may be incurred by the activity of *Bacillus denitrificans*, which oxidizes nitrogenous compounds to molecular nitrogen.

Although some green plants (*e.g.*, the potato) can absorb the

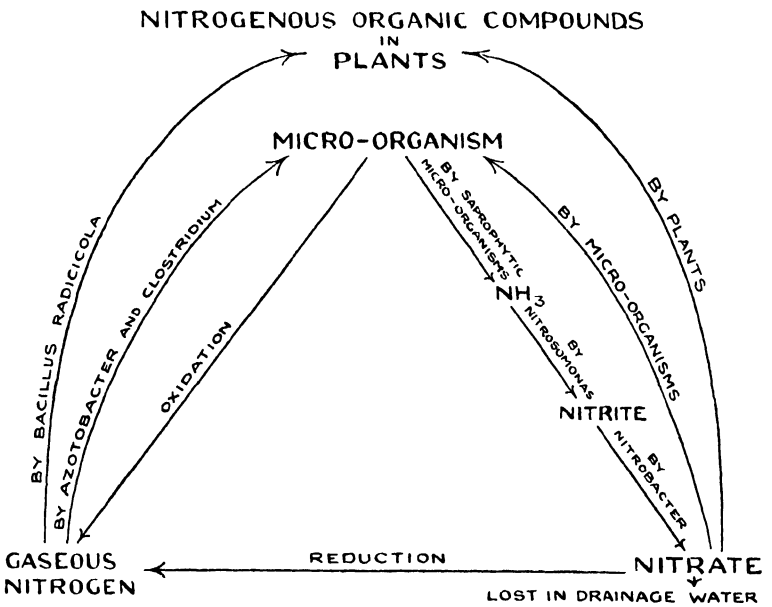


FIG. 7. The nitrogen cycle. (From Russell (*loc. cit.*), slightly modified.)

ammonium salts produced by the ammonification of complex nitrogenous compounds and use them for growth, for most plants the presence of nitrates in soils is essential. The conversion of ammonium salts to nitrates is therefore an all-important phase in the nitrogen cycle (fig. 7). This conversion, which is termed nitrification, is effected in two distinct stages by the nitrifying bacteria. In temperate regions Nitrosomonas oxidizes ammonium salts to nitrites, and Nitrobacter oxidizes the nitrites so produced to nitrates. These bacteria are aerobic organisms, and can grow in the absence of organic foods, *i.e.*, they, like green plants, are autotrophic organisms. They use the energy set free in the oxidations that lead to nitrification,

for the chemosynthesis of carbohydrates from carbon dioxide and water (p. 247). Since only small amounts of nitrate are added by rainfall, and since nitrate is readily leached out of soils, we may infer that under natural conditions a continuous nitrate supply, upon which the growth of green plants depends, is the result of a nutritional process in these chemosynthetic bacteria. These and other (p. 247) chemosynthetic bacteria are, however, in no way dependent on green plants. With the intensive cultivation of land to meet the food requirements of an increasing world population, it became imperative to supplement the nitrates produced by these autotrophic bacteria, and those contained in nitre beds: and one of the most valuable achievements of chemistry and engineering has been the development of a method for fixing atmospheric nitrogen. The amount of nitrate so manufactured has increased in a remarkable fashion during the last forty years, and there is now no danger that the world's food supply will fall short owing to a deficiency of combined nitrogen in soils in a form suitable for absorption by green plants.

C. The Chemical Weathering of Mineral Matter and the Production of the Colloid Clay Complex

Many of the mineral particles produced by mechanical weathering are subjected to chemical weathering under the agency of water, dissolved carbon dioxide, and the organic acids in humus. For instance, felspars, micas, and ferromagnesian and other mineral silicates undergo hydrolysis and produce what has been variously termed the weathering complex, clay complex, zeolite complex, or inorganic soil colloid. Much discussion has centred on the question of the chemical composition of the products of hydrolysis, but no general agreement has yet been reached. It may be definitely stated that no clay has been found to possess the composition of kaolin, which was once supposed to be the essential product of weathering.

Analyses have revealed a wide variation in composition, and few general statements can be made. But it appears that silica and the hydrated sesquioxides of aluminium and iron may account for as much as 90 per cent. of the weathered product. For example, Robinson has reported that the clay fraction of a shale soil from North Wales contained 46·6 per cent. silica, 33·3 per cent. alumina, and 12·6 per cent. oxide of iron. He also found small amounts of basic compounds containing potassium, sodium, calcium, and magnesium. These are exceedingly important components of plant food.

In soils containing sands, and in the coarser silts, unweathered mineral particles may be abundant. Comminuted particles of quartz do not undergo chemical weathering. These, and other mineral fragments which have retained their native composition,

constitute the relatively inert framework of soil. They mingle in moist soil crumbs with the clay complex and organic matter, and serve to give soil an open structure.

D. Soil Solution [1] and the Colloid Complex of Soils

Apart from the elements in water, the essential elements for plant growth that the soil provides are derived from decaying plant remains and the clay complex. Humus yields a mixture of mineral salts (plant ash), and the nitrates, phosphates, and sulphates, produced during decay; and the clay complex yields potassium, calcium, magnesium, and iron. All these elements tend to go into solution in the soil water, to give the "culture solution of the plant." This is retained by absorption and surface attractions in and on the soil particles (fig. 9).

The composition of soil solution is, of course, highly variable. Even over short periods in a given field, the composition is continually changing owing to the activities of plants and micro-organisms; furthermore, evaporation concentrates the soil solution, and rain water dilutes it or leaches out soluble ingredients of the soil. The results of an analysis by Schloesing of a soil solution displaced from a soil, which contained 19·1 per cent. water, are given below. The

SiO_2	Nitric acid	Carbonic acid	CaO	MgO	K_2O	Na_2O	Sulphuric acid	Chlorine	Org. matter
29·1	305	118	264	13·5	6·9	7·8	57·9	7·4	37·5

numbers represent mg./litre. This soil also contained traces of phosphorus and ammonia. The total concentration of solids works out at about 0·08 per cent.

The results of this and other analyses permit the general statement to be made that soil solution is a mixture of the bicarbonates, sulphates, chlorides, nitrates, phosphates, and silicates, of calcium, magnesium, potassium, sodium, and iron. At the dilutions encountered, the salts will be nearly completely dissociated into ions. In soil, therefore, we have a complex heterogeneous system, the solid phase being composed of the hydrogels of the colloid complex, which contain ions in the imbibed water; and the liquid phase consisting of a solution which contains ions. It appears that all the ions excepting those of nitrate and chloride may occur in the solid as well as in the liquid phase.

Nitrates and chlorides occur only in the soil solution. They are

[1] The availability of water in soil solution is considered later (chap. VI, section A).

not chemically absorbed by the colloid complex, and, consequently, if not absorbed by living cells, they are rapidly leached out of soils. The other essential ions are chemically absorbed by the colloid complex, and the distribution between solid and liquid phases for varying concentrations of a given ion appears to be governed by the adsorption equation (p. 458). It is possible therefore that adsorption plays a part in absorption by living cells and by the colloid complex. The power of absorption is an important property of the colloid complex, for it opposes the leaching out by rain water of certain of the essential elements. Solution occurs sooner or later, however, and of all the bases calcium is the one most easily lost (*cf.* p. 96).

Equilibria between ions in the soil are dynamic, and are governed by the distribution of electrical charges. The ions in the colloid complex may be exchanged for others that are contained in the soil solution and bear equivalent electrical charges. For example, if a solution of potassium chloride is added to a soil containing calcium ions in the solid phase, base exchange is effected ; potassium ions are absorbed by the colloid complex, and calcium ions pass out into the soil solution :

$$[\text{Ca-soil}] + \text{added } 2\text{KCl} = [\text{K}_2\text{-soil}] + \text{CaCl}_2$$

Such exchanges are parallel to those which occur when living cells are immersed in saline solutions (p. 88). Now the soil solution bathes the outer walls of the living cells of roots (fig. 9) and of soil micro-organisms. Hence, for a given moist soil in which plants are growing we must, as far as ionic exchanges are concerned, consider the whole system as composed of

[living cells—soil solution—colloid complex].

Thus the exodiffusion of an electrolyte from roots, or from the cells of micro-organisms, would affect the relations between the soil solution and the colloid complex. For example, the liberation of nitrates during nitrification often leads to the elution of calcium ions from the colloid complex. During the growth of plants, however, it is the endodiffusion of electrolytes from the soil solution which mainly directs the transfer of ions. The general tendency will be for electrically charged particles to leave the colloid complex, which will thereby become depleted of plant food. Weathering and the decay of humus will slowly restore fertility, and roots by growing and branching will tap new sources of supply.

CHAPTER VI

THE ABSORPTION OF WATER AND SOLUTES FROM THE SOIL BY ROOTS

A. The Absorption of Available Water

THE experimental system illustrated in fig. 13 can be used to demonstrate that roots absorb water, and to determine the rate of absorption under various conditions. Experiments have shown that temperature has a marked effect on this rate, and that certain plants can be made to wilt simply by lowering the temperature of the water around their roots; recovery may subsequently be effected by raising the temperature. Moreover, if the osmotic pressure (P_e) of the external solution is greater than the suction pressure ($P - T$) of the absorbing cells, water is not absorbed, and plants wilt. As regards the composition of the external liquid it must suffice here to state that since the entrance of water is affected by the state of the protoplasmic membrane, factors such as hydrion concentration, the balance of inorganic salts, and concentration of physiologically active organic solutes, may, at times, exert an influence on the rate of absorption.

Since the corky coverings of old roots are impermeable to water, the plant's water supply must be derived through the functional activity of young roots. Whenever present, root hairs, by extending the surface exposed to the external liquid, will facilitate absorption. We may regard these thin-walled living tubular elements as being in intimate contact with soil water (fig. 9). Russell (*255*) classifies the physical forces, other than the osmotic pressure of the soil solution, that oppose absorption of water from the soil as: (1) Gravity, which acts on all the water, and tends to pull it down to the water table. The effect of gravity is equivalent to a pressure of about 1 atmosphere. (2) Surface forces of capillarity and imbibition, which may range from one up to several hundred atmospheres. Capillarity tends to retain water on the surfaces or in certain of the smaller interstices of soil particles; the water of imbibition, which is sometimes called vesicular water, is held in the interstices of the gels in soil colloids. (3) High surface forces of magnitudes equivalent to several hundred or a thousand atmospheres. So-called gel water, the water imbibed by the molecules of soil colloids, is thus held; it represents only a small proportion of the total water.

The classification of soil water into the sharp groups of gravitational water, capillary water, imbibitional water, and hygroscopic water, was given up when it became apparent that the forces included

above under 1, 2, and 3 " overlap in their operation and that the state of soil water is continuous." The older view that water rises in soils from the water table by capillarity is not accepted by Haines and Schofield (see Keen, *162*), who regard the pore space in soils as essentially cellular, and not capillary. They consider that movements of water are sudden, corresponding with abrupt emptying and filling of the cellular spaces, and that the colloid properties of soil gels exert a powerful controlling influence over this movement. It still remains true, of course, that water will rise to greater heights in glass tubes packed with fine soils (clays and loams), but that more rapid initial movement occurs in the coarser soils (medium loams and sands). These are experimental facts and independent of interpretative theory.

One essential condition for growth is that the roots must absorb water at a sufficient rate to maintain turgor in transpiring members, and to effect turgor enlargement. The first requirement is that the suction pressure of the absorbing cells must overcome the physical forces with which a portion of the water in the soil is held. Water which can be absorbed may be described as available water and such water as is too firmly held for absorption may be termed unavailable water.

The suction pressure approaches zero in absorbing cells which are nearly fully turgid, after wet weather. Under dry conditions the suction pressure is principally determined by the osmotic pressure of root sap, and magnitudes varying from 7 to 20 atmospheres have been recorded for different plants. Obviously, available water must be held by forces of a smaller magnitude.

For a given species grown in different kinds of soil, the actual amount of available water depends upon the magnitudes of the capillary and imbibitional forces with which water is held by the particles in the different soils. It has long been known that the composition of soil exerts an influence on these magnitudes. Thus Sachs experimented on tobacco plants which were growing in different kinds of soils. He allowed the soils to dry, and determined the water content of each soil when the plants began to wilt. The figures he got were : for a sand, 1·5 per cent. ; clay, 8 per cent. ; and a mixture of sand and humus, 12·5 per cent. These figures were supposed approximately to represent the amounts of non-available water in these soils so far as the tobacco plant is concerned. Actually, of course, they represent the amount of water in the soil when absorption does not proceed rapidly enough to prevent wilting. For this reason certain authors denote this amount by the term, the wilting coefficient of the soil. Variations in the same sense have since been found for other plants. One may conclude that the water-supplying power of a soil is limited by the surface forces exerted by clay and the organic

gels in humus. It appears that for quick survey work a rough idea of the amount of unavailable water (and hence also of available water) in any soil may be obtained by assuming that all the water left in soil, which has been dried in air at 15° C., is unavailable. Reckoned as parts by weight of water per 100 parts of dry soil, unavailable water determined in this way varies from under 2 per cent. in coarse sands to over 40 per cent. in peats; and figures between 5 and 10 per cent. have been found for clays and loams. For natural soils composed of diverse admixtures of sand, clay, humus, and, at times, chalk, the amounts of unavailable water are, of course, highly variable.

Maximov (*196*, chap. II) discusses the controversial questions concerning the availability of water for different species growing in a given soil, including the surprising conclusion reached by Briggs and Shantz, viz., that the amount of the available water in a soil is nearly independent of the properties of the plant, and is almost entirely governed by those of the soil.

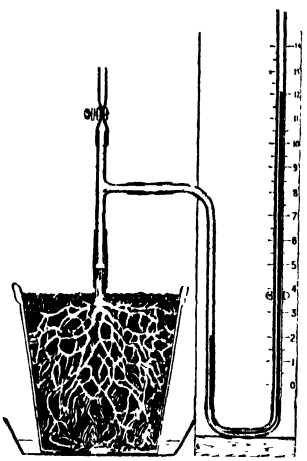


FIG. 8. Apparatus for demonstrating root pressure.

B. Root Pressure and the Lateral Transfer of Water in Roots

Stephen Hales in the eighteenth century demonstrated and measured root pressure. He attached a mercury manometer to the rooted stump of a severed vine shoot (*cf.* fig. 8), watered the plant well, and, from the difference in the final levels of mercury in the two limbs of the manometer, concluded that a pressure of 107 cm. of mercury had developed. Since Hales's time measurements have shown that root pressures fluctuate widely. They may be lower than 1 cm. mercury. Until recently values higher than 200 cm. mercury were held to be exceptional, but White (*333*) has stated in a preliminary report that he has found pressures up to 10 atmospheres (*i.e.*, 760 cm. mercury) in excised roots growing in water cultures.

For a given plant seasonal periodicity has been observed. Wieler concluded from his experiments that (*a*) most hibernating trees lose the power of exuding water for a certain period during the winter, and (*b*) maximal exudation occurs in the spring, but not necessarily when buds are sprouting or new roots forming. Furthermore, it has been found that root pressure may decrease to an extremely low value by the time that transpiration becomes vigorous owing to the production of leaves.

External conditions may affect root pressure at a given season. For example, it is governed by the conditions (water supply, temperature, etc.) that influence absorption. Moreover, certain experimental facts indicate that the process is dependent on a secretory activity of protoplasm, for which respiratory energy must be available. Thus Wieler discovered that on inhibiting respiration,

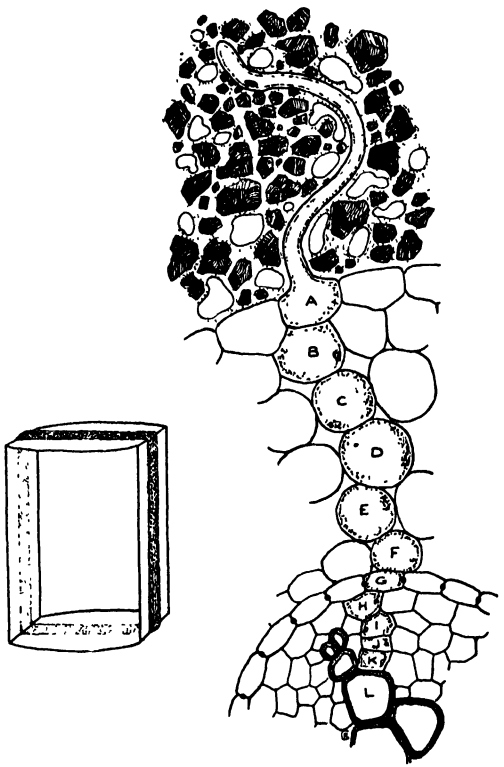


FIG. 9. Diagrammatic representation of the contact made by soil solution with a root hair (A) and with soil particles (from Sachs, modified), and of a transverse section through a root (from Priestley, *235*). Note the suberized thickenings on the radial walls of the endodermis (G) as seen in section, and the whole Casparian band in the drawing of a single endodermal cell.

by cutting off the oxygen supply or anæsthetizing with chloroform, root pressure ceased.

The lateral transfer of water from the soil solution has been a subject of some research and much discussion. For lack of reliable experimental data all we can do at present (see Stiles, *291*) is to infer from the fact that water moves from soil solution to tracheæ the existence of regular suction pressure gradients in the tissues of roots

(say from A to L in fig. 9). Anomalies in the region of the endodermis and pericycle were reported by Ursprung and Blum (see Thomas, *307*). They suggested that the pericycle might act as a combined suction and force pump; but these anomalies have since been attributed to the unsatisfactory methods they used for evaluating from the behaviour of isolated tissue the suction pressures that existed in the living plant (Ernest, *83*, Dixon, *76*).

Thoday (*300*) has pointed out how the living cells in the absorbing region of a root may, layer by layer, gradually become fully turgid under what we here term a suction pressure gradient. In the fully turgid state the living cells can absorb no more water. Then, as Atkins (*7*) had earlier suggested, one may regard the cortical cells as a single complex semipermeable membrane, on the outside of which is the soil solution (fig. 9), and on the inside the solution in the xylem vessels. The force with which water is drawn in would then solely depend on the difference between the osmotic pressure of the xylem sap, and that of the soil solution. Priestley (*235*) incorporated these notions in the hypothesis he put forward to account for the lateral movement of water and the development of root pressure. He clearly formulated the central problem of accounting for a supply of solutes to the xylem at a sufficient rate to maintain the sap at a higher osmotic concentration than that of the soil solution. As a result of his anatomical and experimental investigations he came to the conclusion that the maintenance of this supply of solute required: (*a*) a functional endodermis with impermeable radial and transverse walls (such as would be ensured by the Casparian band (fig. 9)), which prevents the outward leakage of solutes; (*b*) the constant diffusion of solutes into the xylem sap through relatively permeable protoplasts within the endodermal cylinder. Evidence was obtained in his school that dyes penetrated cells near the xylem vessels relatively rapidly; and chemical analysis showed that, in the vine, organic solutes (particularly disaccharides and monosaccharides) and inorganic solutes were present in the xylem sap. The concentration of the latter kept fairly constant, but that of the organic solutes fluctuated; exudation pressures fluctuated correspondingly. Priestley concluded that organic solutes filtering into the xylem vessels, through the neighbouring living parenchyma, are "more directly responsible for the osmotic pressure effective in causing the flow of sap."

It has evidently been widely held that osmotic suction plays an important part in the lateral movement of available water from soil solution to the living cells bordering xylem vessels, and that the unilateral exudation of water from the living cells is also an osmotic phenomenon. But it must not be forgotten that the secretion from a living cell into a dead xylem-element resembles other secretory

processes occurring in plants and animals in being dependent on oxygen uptake, and, presumably, therefore, on respiratory energy. Moreover Bennet-Clark's doubts about the adequacy of the classical osmotic theory (p. 78) touch the question of the lateral movement as well as that of the absorption of water.

C. The Absorption of Solutes

During their growth period, roots continually tap fresh regions of soil, and absorb ions from the mineral salts in the soil solution, and hence from the colloid complex (p. 99) produced by chemical weathering and the decomposition of organic remains. It is no longer held that roots significantly help in preparing their own mineral food, for it appears that they do not secrete organic acids, and such respiratory carbon dioxide as is produced has only a trifling solvent action.

Absorption was for a long time regarded as a purely physical process, each particle diffusing independently of others in the continuous liquid medium of soil solution and plant sap from regions of higher to regions of lower concentration.' On this view any ion occurring in the soil solution is absorbed by plants, provided cell membranes are permeable to that ion (*cf.* p. 79). There is no evidence that ions are passively absorbed with the water taken in by osmotic suction. Indeed, physical experiments (p. 467) have shown that solute and solvent particles may be moving simultaneously in opposite directions. Moreover, in physiological experiments on young barley roots and Cucurbita roots, both of which have a high capacity for absorbing solutes, the rates of absorption of potassium and bromide ions from a well aerated solution of potassium bromide were found to be independent of the rates of absorption of water (Hoagland, *134*). Although in a first analysis the absorption of water and solutes should be regarded as independent processes, the possibility is not denied that solute molecules and water may be absorbed and move across parenchyma in close association ; but our knowledge of such complex mobile units is not yet sufficiently definite for their nature and the control of their movements to be considered here.

Under favourable conditions for growth, molecules and ions of the essential elements are present in the colloid complex, and are continually liberated into the soil solution. Diffusion across permeable membranes into and through the plant then takes place. It has been suggested that solutes also migrate in the wet cell walls. Thus diffusible ions travel towards regions where they are consumed in metabolism or removed from the medium of diffusion by adsorption or in some other way, including that obscure physiological way which causes ions, such as those of potassium, to accumulate in high concentrations in vacuoles. Removal by leakage into the transpira-

tion stream or the phloem slime will have the same effect. In passing we note that the mechanism by which electrolytes that have accumulated in living cells leak into xylem vessels is not understood. Accordingly we do not yet know how conditions necessary for the generation of root pressure originate (p. 104). As Prevot and Steward have pointed out, it is an unsolved puzzle why these same accumulated electrolytes, which in the intact plant can move upward in the shoot, are not removed from healthy cells by washing them with the distilled water.

As long as a plant is living and as long as chemical change goes on in the soil, there can be no equilibrium for any of the essential ions, since the continual production in the soil (the source of ions) and consumption in the plant (the sink for ions) will maintain diffusion gradients. What these gradients will be for a given soil at a given time will depend upon the nature of the metabolic and other processes going on in the plant. Different demands are made by different plants at a similar state of development, and by a single plant at different stages of development. The analysis of the apparent selective—or preferential—absorption by different plants would require a profounder insight into the physical and metabolic processes than we at present possess.

Clearly the experimental results obtained by Hoagland and Davis, and by Steward *et al.*, suggest that a complete description of the absorption of solutes by roots, or by the submerged shoots of water plants, cannot be given at present in purely physical terms. Solutes are sometimes absorbed against a concentration gradient; accumulation of a salt in a plant tissue may be determined by the tissue's powers of growth and development; and the intensity of respiration may control the rate of absorption. Recent work has emphasized the importance of a sufficient supply of oxygen (and, incidentally, therefore, of good aeration of soil) for roots to show their maximum power of absorbing solutes. Many years ago Stiles and Jørgensen (*296*) observed that aeration of water culture solutions caused an increased yield of barley and balsam (though not of buckwheat), and suggested that growth inhibitions resulting from poor aeration might be attributed either to lack of oxygen or to injurious concentration of carbon dioxide. Hoagland and Broyer (*135*) have reported that the absorption of potassium, nitrate, and halides, by excised barley roots was promoted by increasing the oxygen concentration of the culture solutions up to some value equivalent to that found in water standing in contact with air containing rather less than ten per cent. oxygen. Describing more recent work, Hoagland (*134*) states that a small amount of oxygen must be supplied to barley roots before they will begin to accumulate any ions against a gradient, and that, until it attains a relatively high concentration, such as is not usually

reached under natural or experimental conditions, carbon dioxide does not prevent accumulation. Steward, Berry, and Broyer (*286*) recorded a similar effect of raising oxygen concentration on the power of potato roots to absorb potassium bromide. These authors note and discuss the interesting fact that for roots maximum absorption appears to occur at lower oxygen concentrations than those which Steward *et al.* (see p. *92*) found necessary for maximum absorption by discs of storage tissues. Altogether it appears that the peculiar activities of protoplasts may exercise control over the absorption of solutes so as to mask the operation of physical laws of diffusion and of membrane equilibria (*e.g.*, the Donnan equilibria), which, according to classical views, primarily determine the behaviour of plants towards substances in solution.

CHAPTER VII

TRANSPIRATION

A. Introduction

TRANSPIRATION is the giving off of water vapour from the surface of a plant. The phenomenon is readily demonstrated by showing that drops of water condense on a bell jar covering a potted plant

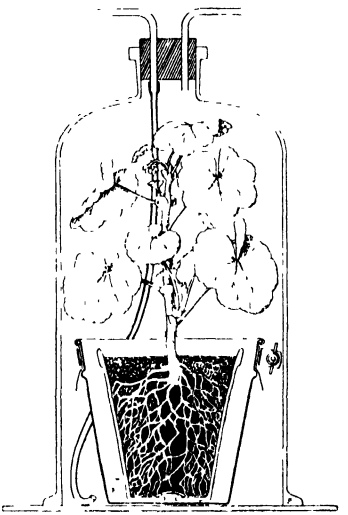


FIG. 10. Apparatus for demonstrating and measuring the transpiration of potted plants. Water loss from the surface of the pot is prevented by encasing the pot in an aluminium shell, and from the surface of the soil by covering, as shown, with rubber sheeting attached to the base of the stem, and held by a clamped band to the aluminium shell. Water should be placed in the aluminium shell at the beginning of the experiment. The presence of inlet and outlet tubes permits the apparatus to be used for measuring transpiration by absorbing the water given off (p. 113).

before they appear on the surface of the plant (see fig. 10). When the outside air is unsaturated all shoot systems lose water by transpiration, and the rate of loss is often high. Thus it has been estimated that in the growing season the Egyptian cotton crop loses 50 tons of water per acre per day, or 3 pints per plant per day. Secretion of liquid water may occur in saturated atmospheres, as may be demonstrated by leaving oat seedlings under a bell jar for a period under conditions favourable for the absorption of water.

High rates of transpiration may be a real menace to the well-being

of land plants, for the maintenance of turgor and the possibility of growth depend upon the absorption by the roots and the conduction to the transpiring organ of a sufficient supply of water. In the lives of most plants there are critical dry periods in which turgor is lost. During prolonged spells of drought the growth rate will be reduced, and the wilted shoot systems may become injured beyond repair.

The structure of plants renders transpiration inevitable. In the first place, although cuticularized and suberized dermal coverings serve adequately in restricting water loss—the fact that a land flora exists is sufficient testimony to their general efficiency—they are not completely impermeable to water. Water loss through cork may be neglected,[1] but loss from epidermal cells through the cuticle is often considerable and is termed cuticular transpiration. The presence of lenticels in cork tissue and of stomata in cuticularized epidermal layers makes possible gaseous exchanges between the living cells in the interior and the outside atmosphere (see p. 153), but inevitably leads to the loss of water vapour by diffusion from the intercellular spaces. The amount which diffuses through the lenticels is negligible, but, in unsaturated air, there is continuous diffusion of water vapour out through open stomata. Stomatal transpiration, then, is chiefly responsible for the loss of water from shoot systems, and is the price shoot systems have had to pay for possessing facilities for gaseous exchange. This price may at times severely tax a plant's resiliency. Hence it is important to realize that through stomatal movements plants possess means of regulating stomatal transpiration. The night closure of stomatal apertures when photosynthesis is in abeyance conserves water without hindering a vital gaseous exchange (see also p. 153). For the short periods involved there is always sufficient oxygen for respiratory purposes in the air contained in the intercellular spaces.

It has often been debated whether transpiration, which is a necessary result of the plant's structure and is fraught with so many harmful consequences, serves any useful purpose. One suggestion is that transpiration may facilitate the movement of solutes. The experimental evidence is conflicting. Thus the results of analyses indicate that the concentration of minerals is sometimes, but by no means always, highest in those regions which have been transpiring most vigorously. Hoagland (*134*) has described an experiment, carried out under conditions favourable for the absorption of solutes, in which the rates of absorption by and distribution of solutes through the plant used were independent of the rates of the absorption and movement of water and of the rate of transpiration. On

[1] Since whole potatoes lose weight very slowly and peeled potatoes quickly, we may infer that even a dermal tissue composed of cells with thin suberized walls and less than a dozen cells thick effectively reduces the rate of water loss.

theoretical grounds one might expect the passive carriage of solute molecules in the conducting tracts of the xylem to be governed by the rate of movement of sap, and hence by the rate of transpiration. But there is no evidence to suggest that the rate of movement of solutes through living tissue is affected by the rate of passage of water. Indeed, the movements of water and solutes are governed by different laws, and in a given tissue solute and solvent may move over a given period in opposite directions (*cf.* parallel physical systems, p. 467).

A second suggestion is that transpiration is mainly responsible for keeping below injurious levels the temperatures of various coloured shoot systems exposed to light. For instance, in one typical experiment it was estimated that seventy per cent. of the light energy absorbed by insolated green leaves was used in transpiration, *i.e.*, was converted into the latent heat of evaporation of water. Only one per cent. was used in photosynthesis. The remaining twenty-nine per cent. caused the temperature of the leaf temporarily to be higher than that of the air, but as a result of convection and conduction of heat in and radiation from the plant, the temperature of the leaves once more became the same as that of the environment. Furthermore, direct measurements have shown that the temperatures of feebly transpiring plant members exposed to the sun may rise considerably above the temperature of the environment. Temperatures higher than 50° C. have been noted in the succulent leaves of desert plants. These plants appear not to be so susceptible to heat injury as are the leaves of mesophytes. Under conditions when mesophytes transpire feebly, as in greenhouse plants growing in saturated atmospheres, the leaves may suffer injury from overheating by the sun's rays. Consequently it has been argued that the cooling effect of transpiration may on humid sunny days save certain plants growing in the open from injury.[1]

B. Experimental Methods for Measuring Transpiration and the Expression of Results

The measurement of transpiration. (i.) *By absorption of the transpired water vapour.* For certain purposes (*e.g.*, the comparison of the transpiration of the upper and lower surfaces of a given leaf), the time taken for dry cobalt chloride paper, placed against a transpiring surface, to change from a standard shade of blue to a standard shade of pink, may be conveniently used as a measure of transpiration. The paper should be protected from atmospheric moisture by a glass plate or mica sheet, and moist air should be prevented from entering at the joins by sealing these with vaseline. Alternatively, changes in weight of calcium chloride tubes

[1] For a fuller discussion of the functions assigned to transpiration, and of Ivanov's suggestion that transpiration maintains an optimal turgor in growing shoots, the reader is referred to Maximov (*196*, p. 102).

affixed by wax or gum to the transpiring surface may be measured. The objections that have been raised against these methods are that the cobalt chloride paper shades the transpiring member, and that the calcium chloride desiccates the air in contact with the surface under investigation. Freeman's method meets these objections. Moist air is drawn from a reservoir in two streams moving at the same slow rate. One stream should first pass over the transpiring organ, suitably secured in a covering of glass, mica, or other translucent waterproof covering, and then through weighed absorption tubes containing calcium chloride, or phosphorus pentoxide. The other stream should pass directly into weighed absorption tubes. From the differences between the increases in weight of the absorption tubes in the two streams, the rate of transpiration can be calculated.

(ii.) *Loss in weight owing to transpiration.* Using Freeman's method again with two streams of moist air, but this time with a whole encased potted plant under a bell jar (*cf.* fig. 10), experimenters have found that the weight of water absorbed by the calcium chloride or phosphorus pentoxide is approximately equal to the loss in weight of the encased potted plant. It appears, therefore, that, under conditions favourable for transpiration, changes in dry weight of the plant (see p. 360) over short periods in the growing season are negligibly small when compared with the weight of water lost. A simple and satisfactory method for determining the rate of transpiration is thus available, and has very often been used in field experiments. Experimental systems are set up which can lose water by transpiration only, and are periodically weighed in order to determine the amount of water lost. For laboratory experiments, systems like that illustrated in fig. 10 are customarily used; in field experiments plants have been grown in glazed pots. Analogous systems can in a variety of ways be set up for detached leaves and cut shoots, and suggestive results have sometimes been obtained simply by weighing at intervals unwatered plant members as they dried in air.

(iii.) *The rate of absorption of water by transpiring members as an indirect measure of water loss.* The potometer (fig. 11) has frequently been employed in researches upon transpiration. Actually this instrument is used to measure the rate of absorption of water by a cut shoot. It can be shown in a variety of ways, however, that the rate of absorption is frequently governed by the rate of water loss. Thus the rate of movement of the bubble in a potometer is reduced by covering with vaseline the surfaces of the transpiring leaves. Moreover, alterations of temperature, humidity, light intensity, and rate of air movement (three factors which, as we shall shortly learn, influence transpiration), affect the rate of absorption. When there is a constant ratio between transpiration and absorption, but then only, the potometer may be used to evaluate variations in the rate of transpiration under different physiological conditions. Shoots for potometer experiments should be cut under water and kept under a bell jar for at least twelve hours before use, with their cut ends in water.

The expression of the results of measurements. (i.) *The intensity of transpiration.* In physiological experiments on transpiration the same transpiring shoot is as a rule under different environmental conditions, and it is sufficient to express transpiration by a number representing the amount of water lost in unit time for the given shoot system. For certain purposes, however, attention must be paid to the extent of the transpiring surface, and the term *intensity of transpiration* has been given to the rate at which water is lost by unit surface of the transpiring organ. The area of the transpiring surface is best measured with a planimeter, but approximate measures can be obtained by cutting out paper areas equal to those of the

transpiring members, and weighing the pieces of paper. The area of the transpiring surface may thus be calculated if the weight of unit area of the paper is known.

In experiments on the intensity of transpiration, transpiring surfaces not under investigation should be covered with tinfoil or vaseline.

(ii.) *Relative transpiration.* A difference (between T_1 and T_2, say) in the rate of transpiration of a given shoot system during two successive periods

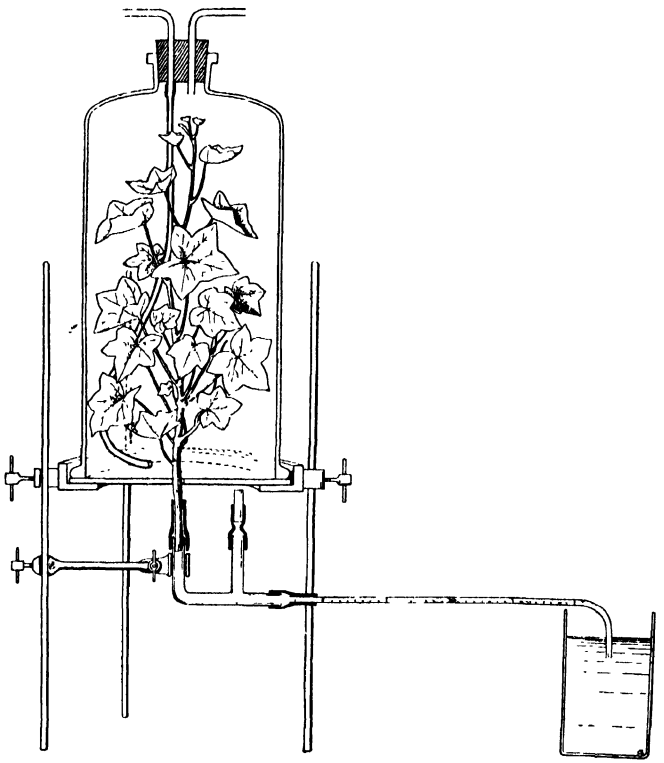


FIG. 11. The potometer, for measuring the relative rates of absorption of water by a given cut leafy shoot under different environmental conditions. The glass pieces must first be completely filled with water, then a small air bubble is introduced into the horizontally placed graduated tube, and the rate of travel of the bubble over a selected distance is measured. The solid glass rod is used to adjust the position of the bubble in the graduated tube.

must be attributed to alterations in the environmental factors that affect evaporation (*e.g.*, temperature, humidity, or wind velocity), or in the internal factors (*e.g.*, width of stomatal apertures, or water-holding power of cell walls) that have a regulatory effect on transpiration. Simultaneous alterations in the evaporating power of the atmosphere (*e.g.*, from E_1 to E_2) are readily determined by means of evaporimeters or atmometers (see Maximov, *196*). Livingston's concept of relative transpiration has been used in attempts to assess changes in the regulatory power of a plant over

its own transpiration. Relative transpiration is given by the ratio of the rate of transpiration per unit area (T) to the rate of evaporation per unit area (E).

Livingston supposed that were T_1/E_1 found to be equal to T_2/E_2, the changed transpiration rate could be entirely attributed to alterations in the evaporating power of the atmosphere, but that inequality in the two ratios would indicate that the plant's resistance towards water loss had altered. Thus he made investigations on the influence of light on the relative-transpiration of certain desert plants, and concluded that internal regulation by these plants is more effective at night than by day. For example, he found that the relative transpiration of a certain species of Euphorbia showed a maximum during the day of about 0·07, and a minimum at night of less than 0·01.

For the use of relative transpiration as a valid measure of internal regulation, the rate of the *evaporation component* of transpiration must vary proportionately with the rate of evaporation from the evaporimeter or atmometer, under changing environmental conditions. Unfortunately it is probable that evaporation from physical instruments may at times be more strongly affected by changes in environmental factors, particularly wind velocity, than is the evaporation component of transpiration. And it has been stated that alterations in T/E might thus measure the altered responses of the physical instrument and not those of the plant. Certain authorities maintain, however, that, provided the wind velocity remains constant, changes in relative transpiration actually do reflect changes in the plant's regulatory power.

C. Cuticular and Stomatal Transpiration

Hypostomatal leaves serve for experimental investigations on the efficiency of cuticle; and on the relative magnitudes of cuticular and stomatal transpiration. The transpiration from the upper and lower surfaces of a given hypostomatal leaf may be readily compared (section B, 1 (i.)), or the percentage loss in weight, over a period, of a sample of hypostomatal leaves with their under stomatal surfaces vaselined may be compared with that of another sample with vaselined astomatal upper surfaces (section B, 1 (ii.)). Experiments with a potometer (section B, 1 (iii.)) may also prove instructive, if a shoot bearing hypostomatal leaves is used. Under constant external conditions, measurements should be made of the relative rates of absorption of water by the untreated shoot, by the shoot when the upper surfaces of the leaves have been covered with vaseline, and by the shoot when both surfaces have been vaselined.

Similar experiments have been performed with shoots bearing amphistomatal leaves, in order to determine whether the rate of transpiration from the surface of a given leaf bears a quantitative relation to the number of stomata on that surface. Moreover, suggestive results have been obtained by varying the conditions around a given shoot system so as to permit measurements of changes in transpiration when stomatal apertures are widening or narrowing.

The experimental results indicate that some water is evaporated from the outer walls of most epidermal cells and escapes as vapour

through the cuticle. The rate of such cuticular transpiration may be considerable in young leaves, and even in older leaves when the cuticle remains thin; but thick cuticle is very efficient in preventing water loss. Additional layers of wax (as, for example, in the apple), or of resin (as in horse-chestnut buds), sometimes add to the efficiency of cuticularized dermal coverings.

Transpiration is usually more vigorous from the stomatal than from the astomatal surface of a given hypostomatal leaf, and correlations have been found to exist between the numbers of stomata on and the rates of transpiration from the upper and lower surfaces of a given amphistomatal leaf. Although the simplest and most probable explanation of these differences in rates of water loss from opposite surfaces of the same leaf is that stomatal exceeds cuticular transpiration, it should be realized that cuticular transpiration is also taking place from the stomatal surfaces, and that it may be greater there than from the astomatal surfaces. Further evidence must therefore be sought for the general predominance of stomatal over cuticular transpiration. This is given by the results of measurements of the diurnal transpiration of leaves whose stomata are closed during the night. In one experiment on maize it was found that, after making allowance for change in the evaporating power of the atmosphere, the direct effect of darkening the leaves was to reduce transpiration to one-twelfth of the maximum daytime value. This reduction may have been in part brought about by a diminution in cuticular transpiration, which, of course, persists during the night. Experimental data on the direct effect of light on the evaporation of water from living cells show, however, that cuticular transpiration, at the very most, would not have been reduced by more than one-half by darkening the leaves. Consequently, we may infer that the chief cause of the reduction was the cessation of stomatal transpiration when the apertures closed. It would follow that stomatal transpiration was, in the daytime, much more vigorous than cuticular transpiration. Thus, assuming that stomatal transpiration ceased completely, and that cuticular transpiration was actually reduced to one-half on darkening, we can calculate the ratio, cuticular transpiration/stomatal transpiration, for the leaves transpiring in the light. In the daytime, cuticular transpiration + stomatal transpiration = 12 units; at night, cuticular transpiration (day value)/2 = 1 unit. Hence cuticular transpiration (day value) = 2 units, and stomatal transpiration (day value) = 10 units. Consequently, stomatal transpiration would be five times greater than cuticular transpiration during the day.

It appears that when stomata are wide open, stomatal transpiration on the average accounts for eighty per cent. of the water loss from the surfaces of leaves. There is, however, much variation from leaf

to leaf. For young leaves with thin cuticle and partly developed stomata, cuticular may exceed stomatal transpiration. On the other hand, stomatal transpiration accounts for nearly all the water lost from a fully developed leaf with a thick cuticle.

Finally we note that whereas cuticular transpiration simply consists in the evaporation of water from the wet walls of epidermal cells, stomatal transpiration proceeds in several stages. When the stomata of a leaf placed in unsaturated air begin to open, water vapour will diffuse out from the intercellular spaces through the stomatal apertures at a rate which, for a turgid shoot, will depend upon the diameter and length of the pores, and the pressure gradient (see chap. X, section D). The result of this diffusion will be an increase in the saturation deficit in the intercellular spaces. Hence the evaporation of water from such wet cell walls as abut upon these spaces will be promoted. As long therefore as stomata remain open and the outside air remains unsaturated, water will be lost from the leaf as a result of the combined action of these two component processes of stomatal transpiration, viz., evaporation and diffusion.

D. The Rate of Transpiration

The interpretation of the results of field experiments. Briggs and Shantz, and Maximov (see Maximov, *196*) have independently collected strong evidence, for a wide range of plants, that transpiration under natural conditions often marches with solar radiation. In this respect they found that transpiration resembled the physical process of evaporation, which was simultaneously measured. Their results showed that during the day both transpiration and evaporation marched with the saturation deficit, and they concluded that solar radiation affected the evaporating power of the atmosphere by influencing the saturation deficit. In Maximov's experiments, however, wind produced a marked effect on evaporation, but no apparent effect on transpiration. But there is other evidence that at times transpiration is markedly increased by air movement.

The term *saturation deficit* refers, of course, to the difference ($P_t - P_e$) between the saturation pressure (P_t) for water vapour in air at the temperature of the experiment, and the actual pressure of water vapour (P_e) in the air at the time of the experiment. Clearly the two factors which determine the magnitude of the saturation deficit are temperature and humidity, for the saturation pressure P_t is solely governed by the temperature, and P_e is a measure of the humidity.[1]

[1] (*a*) The difference between saturation deficit and humidity should be carefully noted. Consider, for example, two separate localities where the humidity of the air is the same and the temperature different. The saturation deficit would be higher in the warmer locality.

(*b*) Another point to note is that saturation deficits may be higher on cold dry days in the winter than on warm moist days in the summer.

Presumably, solar radiation affects saturation deficit by governing the temperature of the atmosphere.

In the field experiments under discussion, the close correspondence of transpiration to evaporation did not continue for all the plants during the hours of darkness. As the temperature, and hence the saturation deficit, diminished, both evaporation and transpiration decreased. In some of the plants, however, transpiration fell relatively far more than evaporation, *i.e.*, relative transpiration (T/E) was higher during the day than the night. It was inferred that the powers possessed by these plants of regulating transpiration are relatively more effective after sunset. In these experiments stomatal closure induced by the absence of light was probably responsible for such falls in relative transpiration as were observed.

The results of these and of other important experiments under natural conditions have served to indicate that transpiration for a given plant is a process which may be affected by many separate factors, viz., the *external factors*, (*a*) saturation deficit (which is the resultant of the effects of temperature and humidity); (*b*) wind velocity; (*c*) light intensity; and (*d*) the water content of the soil; and the *internal factors*, (*e*) the dimensions of stomatal apertures; and (*f*) the water-holding power of transpiring cells. We may suppose that the rate of transpiration at any time will be limited either by the rate of the evaporation component, or by that of the diffusion component of the whole process. All the factors listed above can affect evaporation, and all, excepting (*f*), diffusion. The influence of (*c*) and (*d*) on diffusion is indirect, and is exerted on (*e*).

The influence of external factors on the rate of transpiration. For the consideration of the effect of external factors we must postulate that the plant itself is well supplied with water, and that the stomata are wide open, *i.e.*, internal factors are tending to facilitate and not to hinder transpiration. Under constant light intensity in still air, transpiration is governed by the saturation deficit. In a shoot well supplied with water the pressure of water vapour in the intercellular spaces may be represented by P_t, the saturation pressure of water vapour at the temperature t. Diffusion of water vapour will inevitably take place through the wide-open stomata so long as P_t exceeds P_e, the actual pressure of water vapour in the outside air. The rate of diffusion will be proportional to $(P_t - P_e)/L$, where L is the mean length of a stomatal pore. For a given shoot system, L is constant; consequently transpiration would be proportional to $P_t - P_e$, the saturation deficit. The rate of transpiration could be increased at constant temperature by lowering the humidity, or at constant humidity by raising the temperature.

Further, for a given shoot system well supplied with water, with wide-open stomata, and kept in air at a constant saturation deficit,

there is some evidence that transpiration may rise in response to an increase in light intensity or to the occurrence of gentle air movements.

(Light energy when absorbed by plant members is largely converted into heat energy. The temperature of illuminated leaves thus tends to rise, and transpiration is promoted.) But light energy may, without conversion into heat energy, occasion changes in the rate of transpiration. It has been suggested that increase of light leads to an increase in the permeability of protoplasm, and hence to an increased rate of supply of water to the wet walls. It should be noted that we have ruled out any possibility of stomatal movements under the influence of light by postulating that the stomata were wide open all the time.

In practice it is not easy to ensure during an experiment constant sizes of open stomatal apertures. In order to eliminate the effect of stomatal movements, F. Darwin covered the lower surface of a hypostomatal leaf (*e.g.*, cherry laurel) with vaseline, and arranged for communication with the outside atmosphere by making small slits between the veins. This work has been repeated by Henderson (*127*), who avoided some possible sources of error in Darwin's experiments.

Air movements remove from the surfaces of leaves, particularly in the vicinity of the stomata, layers of air richer in water vapour than is the main body of the air. In still air the presence of these layers would reduce the diffusion gradient along the stomatal pores. Consequently the rate of diffusion through the stomata would be diminished, and transpiration retarded. Strong air movements, by causing branches to sway, lead to the expelling of water vapour in mass. Secondary effects, as a rule, result during windy weather, *e.g.*, stomata often tend to close, and thus the intensity of transpiration diminishes.

The influence of internal factors on the rate of transpiration. Livingston, by showing that relative transpiration (T/E) fluctuates during the course of twenty-four hours, has provided us with the clearest demonstration that transpiration for a given plant is not wholly governed by the evaporating power of the atmosphere. There are internal factors which appear to contribute to the power a plant possesses of regulating its transpiration. Of these factors, (i.) the width of stomatal apertures, and (ii.) what has been termed the water-holding power of cells, have received the greatest attention. It is difficult to arrange ideal conditions for the separate study of these two factors. When stomatal control is to be studied the transpiring shoot should be well supplied with water, in order to keep the water content of the cells as high as possible. In contrast, for the study of controls other than stomatal, measurements have been made of the transpiration of shoots which were incurring an increasing water

deficit, or were wilting, owing either to an inadequate water supply or to the high evaporating power of the atmosphere. Although in such experiments there is no known way of keeping constant the size of stomatal apertures, inferences concerning the water-holding power of the plant may sometimes be made from the data gathered. For instance, it was observed that under constant external conditions the transpiration of a wilting leaf diminished while the stomata showed a tendency to open. Hence it was concluded that the leaf had exercised over its transpiration a control which was independent of stomatal movements. Maximov studied this phenomenon by working in the afternoon and evening with plants (*e.g.*, maize) whose stomata closed at about noon, and found that transpiration, which was, of course, entirely cuticular, was markedly affected by the water supply.

(i.) *The width of stomatal apertures and the rate of transpiration.* The extreme view was once held that transpiration is wholly governed by stomatal guard cells. The results of experiments in which transpiration, evaporation, and stomatal apertures, have been simultaneously measured (for a brief review see Barton-Wright, *17*) have, however, established the fact that the width of stomatal apertures is only one of the restrictive influences, external and internal, which are operative in determining the rate of transpiration. For instance, Loftfield found (*a*) that only cuticular transpiration can occur when stomata are completely shut; (*b*) that stomatal regulation of the transpiration of a leafy shoot well supplied with water usually occurs during the final phases of a closing movement and during the initial phases of an opening movement (thus, in general, stomatal apertures and transpiration increase together early in the day); (*c*) that stomatal regulation during closure often has the functional value of facilitating the recovery of healthy turgor by shoots which have shown a tendency to wilt (thus closure by night aids the making good of water deficits incurred by an excess of transpiration over absorption by day); and (*d*) that when stomatal pores are wider than one-half of their maximum width, either some other internal factor or an external factor (*e.g.*, saturation deficit) governs the rate of stomatal transpiration to an ever increasing extent.

Since the laws governing the diffusion of gases through minute apertures (p. 157) apply also to the diffusion of water vapour, it follows that when the rate of stomatal transpiration, for a given leaf, is restricted by the size of stomatal apertures, it will be the *mean diameter* of the pores and not their mean area which will govern the rate. The mathematical relations that have been developed from the diameter law indicate that, for many leaves, far more water vapour could diffuse in unit time through fully open stomata than is ever actually lost by transpiration. This supports Loftfield's contention that stomatal regulation is not encountered when stomatal

pores are wider than one-half of their maximum width : other factors then limit the rate of transpiration. For narrower pores, when stomata exert a regulatory influence, the fact that the transpiration is governed by a diameter law is not an advantage to the plant, since the rate of decrease of transpiration during closure is less than it would be were diffusion governed by an area law. For example, for circular apertures, halving the diameter would approximately halve the rate of transpiration, and not reduce it to one-quarter, as would result under an area law.

(ii.) *Water-holding power of cell walls, and the rate of transpiration.* Livingston and others have reported that relative transpiration (T/E) sometimes falls during the course of the day before stomatal closure begins, and they have consequently inferred that plants are capable of a non-stomatal foliar regulatory response. On the other hand, this fall may have been due, not to regulation by the plant, but to the fact that a new meteorological condition (*e.g.*, increased wind velocity) had an accelerating effect on evaporation from the atmometer used, without perceptibly influencing transpiration, *i.e.*, the decline in T/E was due, not to a diminution in T, but to an increase in E (see also p. 115). Livingston and Brown supposed that a state of incipient drying may be set up when the transpiration rate is high, and that this leads to an increase in the water-holding power of the cells in the transpiring shoot.

Sresnevski's theoretical considerations suggest that foliar regulation of this type would be a purely physical phenomenon. One may picture the cellulose cell walls of plants as porous fabrics which imbibe water from the saturated protoplasts or directly from the conducting tissue-elements in the xylem. If the rate of water supply is high, the minute capillaries in the walls will be filled—and there may even be liquid water on the outside. Evaporation would then occur as from the surface of water in a vessel filled to the brim. But in detached tissues drying in air, or in attached shoots when transpiration is in excess of the supply arriving at the transpiring regions, the outer layers of the cell walls will inevitably tend to dry. Consequently the curved menisci of the fine water columns will gradually approach the protoplasts. Sresnevski pointed out that physical theory supports the view that as water thus recedes in the minute capillaries of cell walls, the rate of evaporation will progressively decrease.

Experiments also lend support to these views. Thus it is well known that the rate of drying out of water-saturated fabrics decreases as their water content falls, and that it is with difficulty that the last traces of water are expelled. Plant tissues behave similarly. For example, the rate of water loss from ripening seeds is at first rapid, but later diminishes; and, after gathering, the mature seeds may

retain water for long periods even when stored in dry air. The drying out of leaves in air illustrates the same principle. Bews found that the average rate of water loss from certain leaves on the first afternoon immediately after cutting was 0·12 grams per leaf per hour. On the next afternoon for the same leaves under the same conditions the rate had fallen to 0·03 grams per leaf per hour. Had the original rate of loss been maintained the leaves would have been quite dry after sixteen hours, but they still contained 0·004 grams of water after three and half days.

Knight (*170*) by an ingenious method investigated the effect of less drastic drying on retentivity. He found that the rate of transpiration

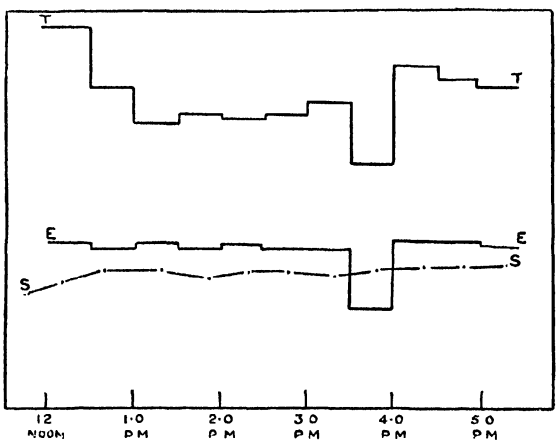


FIG. 12. The influence of water content of the leaves on the transpiration of *Eupatorium adenophorum*. (From Knight, see text.) S = stomatal aperture, E = evaporation (as measured by an atmometer), T = transpiration. The air current was stopped at 3.30 p.m. and started again at 4 p.m.

of a leafy shoot of *Eupatorium adenophorum*, although it was throughout the experiment greater than absorption, diminished when the shoot was exposed to an artificially generated current of air, in spite of the facts that the evaporating power of the atmosphere remained constant, and that the stomatal apertures widened slightly (see fig. 12). The total water content of the shoot decreased by 413 mg., and Knight inferred that the incipient drying represented by this deficit was responsible for the increase in the water-holding power of the cell walls, and hence for the fall in the rate of transpiration. For a short period during the course of the experiment the air current was stopped, and absorption temporarily exceeded transpiration, which fell to a low level. Consequently the water content of the shoot increased during this period. When the air current was started

again, transpiration at once increased, and attained a value that was greater than the rate just before the current was stopped, *i.e.*, when the water content of the shoot was considerably lower. This increase of rate, therefore, provided further evidence that the rate of transpiration bore a relation to the water content of the transpiring shoot.

E. Notes on the Water Balance of Transpiring Members, Water Deficits, Wilting, and Drought Resistance [1]

The total water content of a transpiring member fluctuates daily owing to changes in the relative rates of supply (compounded of rates of absorption from the soil and of conduction in the plant) and transpiration. When supply exceeds transpiration there will be a positive water balance, and the turgor of the living cells will increase. Such a state would favour growth in length, and might be brought about (*a*) by the promotion of absorption and conduction through an abundant supply of soil water, and warmth, or (*b*) by the restriction of transpiration through the narrowing of stomatal apertures, or the decreasing of the saturation deficit.

A negative water balance results when transpiration exceeds supply. The creation of water deficits on balance is due, not to a stoppage of supply, but to an insufficiently rapid supply. Thus they may be brought about (*a*) by the promotion of transpiration through the opening of stomatal apertures, the drying of air, or the rising of the wind; or (*b*) by the retarding of absorption and conduction through the drying of the soil, or the reduction of temperature. Simple experiments on the water balance of plants may be performed with the apparatus illustrated in fig. 13.

Turgor pressures will be reduced in the cells of plant members which are incurring water deficits, and there will be danger of wilting. Drooping leaves are familiar objects in all seasons. Thus in winter, on dry frosty days, water is absorbed extremely slowly, and the leaves of evergreens may wilt; transpiration may be slow, but supply is even slower. We associate wilting in the summer with periods of drought, *i.e.*, with high saturation deficits and soils which lack available water. The old tag "soils physically wet may be physiologically dry" has point here; since it has been found that, for a given plant, the percentage of water that a soil must contain to save the plant from wilting depends upon the nature of the soil. Thus Briggs and Shantz found that what they termed the "wilting coefficient of the soil" (*i.e.*, the water content of a soil, expressed as a percentage of its dry weight, when wilting was observed) might fluctuate for a given plant from less than one per cent. for a dune sand to seventeen per

[1] Suggestive and critical discussions of these important topics will be found in Maximov (*196*), Part III.

cent. for a clay loam. It is not surprising that values for the unavailable water (p. 103) and the wilting coefficient march together in the different soils of the series sands, clays, loams and peats.

The unsatisfactory terms transient wilting and permanent wilting have been used to describe degrees of wilting. Transient wilting can be corrected simply by shading plants so as to reduce transpiration, but root systems do not recover from permanent wilting unless they are watered. We may group these together as reversible forms of wilting, since both are transient and neither is permanent, and use the term irreversible wilting when, even under the most favourable conditions, turgor is not regained.

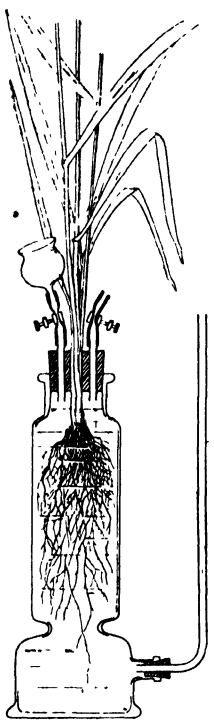


FIG. 13. Apparatus for demonstrating and measuring the rate of absorption of water by roots under various conditions (see p. 102). By determining changes in weight of the whole system, transpiration may be measured simultaneously.

It will be realized that during wilting, as turgor pressures decrease, cells contract. Certain observations suggest that whereas the volumes of the cells of herbaceous plants living in the open may contract considerably before turgor is finally lost, quite small contractions lead to the loss of turgor by the cells of shade plants. Consequently shade plants tend to wilt more readily than plants which normally grow in the open. During prolonged periods of drought, however, all plants are in danger of injury. Resistance to water loss may increase owing to the narrowing of stomatal apertures,[1] and the drying cell wall, or substances (*e.g.*, mucilages) within the cells, may hold water ever more tenaciously; but sooner or later cells in which the water deficit steadily increases will cease to possess turgor. At this stage the cells, which will have contracted to their maximum extent, will be surrounded with air (*cf.* cells which are just plasmolyzed: these are immersed in a hypertonic bathing solution). Further evaporation leads to the contraction of cell contents, and the cell wall, being drawn in with them, becomes folded and wrinkled

[1] Actually during wilting there is a complicated succession of closing and opening movements of stomata. Of course, closure is an effective protective mechanism; but opening, although it may serve to prevent starvation, aggravates the menace of persistent conditions of drought.

(*cf.* plasmolysis, in which the protoplast withdraws from the cell wall, the intervening space being filled with the hypertonic bathing solution). Iljin has suggested that injury and death, which follow irreversible wilting, are caused by the deformation or tearing of the shrinking protoplasts of drying cells, and has thus virtually related all the mechanisms for drought resistance to the necessity of maintaining the structure of living protoplasm. The consideration of problems concerning these mechanisms belongs to the province of ecology.

CHAPTER VIII

THE CONDUCTION OF WATER

A. The Channels and Rate of Conduction

Conduction in xylem tissue. The phrase "up the wood and down the bast" summarizes the views that have long been held concerning the movement of liquid in plants. What is meant is that water, and mineral salts and other solutes, migrate to all parts of the plant in the xylem, and food materials manufactured in green cells are conducted in the phloem to regions of growth and storage. Malpighi's ringing experiments performed in the late seventeenth century indicated that different powers of conduction are possessed by wood and bark. He removed a girdle of bark from a woody stem, and found that, although the growth of roots was inhibited below the girdle (*cf.* pp. 139 and 140), leafy branch shoots developed above. Malpighi thus demonstrated that bark need not be present for the upward conduction of water, but must be present for the downward conduction of food. The importance of xylem in the conduction of water, with which we are at present concerned, was established in the last century, when it was shown that the leaves of a cut shoot (*e.g.*, elder), with the cut end submerged under water, did not wilt rapidly when a girdle of extra-cambial tissues was removed, and a length of pith bored out. Moreover, it was observed that when measures were taken to prevent morbid changes such as drying and rotting in the region of the ring the leafy crown of girdled trees remained alive and grew for a long time. Clearly water must have been conducted in the xylem across the girdled portion of the trunk sufficiently rapidly to replace the water lost by transpiration and to effect enlargement in the growing regions.

Ringing experiments have also shown that older woody tissue may lose the power of conducting water. Thus, when it was observed that shoots wilted above the girdles made by removing bark and the outer regions of wood from the stems of oak, pine, and some other trees, it was concluded that the inner duramen layers cannot conduct water sufficiently rapidly to maintain turgor in the leafy shoots.

Conduction in the lumina of vessels and tracheides. The earlier anatomists ascribed to the woody fibres the function of conducting water, and to the tracheal elements that of providing conduits for the movement of air. But it is now established, and readily demonstrated, that water ascends in the lumina of the vessels and tracheides. Cut leafy shoots, placed in water, wilt if the lumina of the vessels have previously been blocked with gelatine or a wax that has a low melting

point. After occlusion has been effected by placing the cut shoot in the molten substance for a period and then cooling, a thin section should be removed from the base of the stem, so as to ensure that the cell wall of vessels and parenchyma are exposed to water when the cut end of the shoot is submerged. This experiment shows that the residual conducting power of the shoot after blocking the lumina is insufficient to maintain turgor. Hence it follows that water cannot ascend along cell walls (as Sachs believed it could), or through parenchyma, sufficiently rapidly to prevent wilting.

The suppression of conduction by the occlusion of the lumina of vessels may also be demonstrated by applying pressure with a screw-clamp to the stem of a leafy shoot fixed in a potometer (fig. 11). Compression leads to a reduction in the rate of absorption, which may fall to zero, and the leafy shoot wilts. If the screw clamp is then loosened, water is again absorbed. In an experiment of this kind Dixon and Ballard (*76*) have observed that cutting under water a wilted shoot of *Vitis striata* above the clamp, led to elastic recovery in a few minutes, "petioles and tips of stems . . . often moving through 90° in a few minutes." They consider that the water in the lumina of vessels is under tension (see below), which increases during wilting with resulting contraction of the vessel walls, and that this tension is rapidly released as liquid enters the vessels after the shoot is cut under water.

A simple method of demonstrating that water can ascend in vessels is to leave cut transpiring shoots of a herbaceous plant in a solution of a dye (*e.g.*, eosin) for a period, and then to make transverse sections at different levels, and examine under a microscope. The dye, which will be passively carried in the ascending liquid, will stain the walls of the conducting tissue elements, viz., the vessels. In the light of the knowledge gained from other experiments we may assert that the coloured liquid ascends in the lumina of the vessels.

The downward conduction of sap. Strasburger obtained convincing evidence that sap can move downwards as well as upwards in the conducting channels. He observed a pair of trees whose stems, having come into contact, had become organically fused, and severed the stem of one of the trees below the region of fusion. He thus arranged that the leafy branches of the severed tree below this region were dependent on the water absorbed by the roots of the entire tree, and, finding that these leafy branches lived for a long time, concluded that water was supplied to them by an upward movement of sap in the wood of the entire tree, and a downward movement in the basal part of the severed limb of the other tree.

A more recent experiment of Dixon's is also informative. He introduced a dye into the cells of a foliage leaf, and showed that it migrated in the wood vessels into the stem below. Clearly, therefore

in considering the movement of liquid in the wood, we must remember that, in addition to the ascent of sap, downward movement may be occurring simultaneously.

The rate of conduction of sap. Much variation occurs in the rate at which sap moves in the conducting channels of a given plant. The rate is relatively high when a leafy shoot is transpiring freely, and may be extremely low when transpiration is feeble. When, during dry summer weather, turgor is maintained in the cells of growing shoots at the top of a tall tree with a great leafy crown, the average rate over a twenty-four hour period approaches a maximum value.

Sachs obtained numbers representing relative rates of ascent for different plants by watering the roots with solutions of lithium nitrate, and tracing the ascent of this salt by means of a spectroscope. He found that, under the conditions of the experiment, the lithium salt migrated at a rate which varied from 0·2 to 2 metres per hour. Numbers of a similar order have been obtained by measuring the rates of ascent of eosin in cut shoots of various species. The observed rate was, however, higher in stems possessing vessels with large diameters. For example, a rate of 6 metres per hour was measured for shoots of climbing plants (*e.g.*, Bryonia). Clearly, if a given area of conducting tissue is occupied by a few vessels of large diameter, the frictional resistance offered by the tracheal walls would be less than if the area were occupied by a large number of narrow tracheal elements.

In performing experiments on the rate of movement of sap, Farmer (*85*) employed the notion of the specific conductivity of wood, which he defined as " the absolute volume in ccs of water passing through 1 sq. cm. of a 15 cm. length of cut stem or root in fifteen minutes under a pressure head of 30 cm. mercury." The variability of frictional resistance from species to species is illustrated by the fact that the specific conductivities he measured ranged from 0·86 to 95. As might be expected, the specific conductivities were found to be relatively high for trees that can transpire freely.

B. On the Motive Power that Propels Sap

Vital activity of xylem parenchyma not necessary. A comprehensive theory of the ascent of sap must be able to account for the movement of sap, in the lumina of vessels and tracheides, from the absorbing regions of roots to the tops of the tallest trees, at a sufficient rate to replace the water lost by transpiration, and to bring about turgor enlargement in the growing regions. We can therefore at once rule out the idea that atmospheric pressure is the sole agency, for its maximum effect would be to support a 34-foot column of water, whereas a general explanation must account for ascents of 300–400 feet in trees such as the giant Sequoias of California. Furthermore, capillary rise in the *lumina* of vessels is of trifling significance in tall

shrubs and trees, since in narrow vessels of diameter 0·03 mm., the capillary rise would be less than 4 feet.

Many physiologists have favoured the view that the ascent of sap is effected by a sort of pumping action of the living parenchyma in proximity to the conducting vessels and tracheides. Concerning the older theories, Jost (*158*) states that : " the essential point in all these theories is that parenchymatous cells abstract water from one vessel and hand it on to one higher up." Bose advocated a vital theory of the ascent of sap, in which he attributes the ascent to pulsations in the inner parenchyma of the cortex. And there are others who support the view that the ascent of sap is dependent on the vital activity of living cells in or adjoining the vascular systems of root and stem. The majority of physiologists, however, have attached great significance to experiments, such as those of Strasburger, in which it was demonstrated that sap can ascend along lengths of stem which had previously been killed by heat, or with cell poisons such as picric acid.

Strasburger sawed through the base of the bole of a young oak tree, and slung the severed trunk and branches by ropes attached to supports. He then caused the severed bole to be swung so as to bring its cut end into a tilted tub containing picric acid. This poison reached the top of the tree. Eosin was added to the liquid in the tub three days after the beginning of the experiment, and it was observed that this dye was passively carried to the top of the tree in spite of the fact that the picric acid had previously killed the living cells in the neighbourhood of the conducting channels. It was considered that this and other similar experiments demonstrated that sap can ascend in xylem containing no living cells. Applying Occam's razor, " *Entia non sunt multiplicanda praeter necessitatem*," many physiologists have therefore concluded that the actual mass movement of sap in vessels and tracheides of living trees is a purely physical process. Those who hold such views regard these conducting elements as so many pipes along which water is propelled in living trees by forces that are operative either in the absorbing regions of the roots or in the living regions to which water is conducted in the leafy shoot.

Root pressure alone inadequate. In the spring just before buds develop the water that is absorbed from the soil may be secreted into xylem vessels under considerable pressure (p. 104). According to older measurements root pressure may attain magnitudes of 3-4 atmospheres, but the maximum for a given plant is as a rule under 2 atmospheres. It was thought, therefore, that in the spring, when transpiration is low and root pressure is at its maximum, sap may be raised from 50–100 ft., but no more, by forces operative in living cells within the endodermal cylinder of roots. Moreover, the evidence indicated that root pressure rapidly diminishes and may fall to a very

low level when transpiration becomes active, after the unfolding of the leaves. When leafy stems are cut, water does not exude; on the contrary when cut under coloured liquids they are seen to be easily injected both upwards and downwards, indicating the existence in them either of tensions in the sap or of negative gas pressures. Hence it has been argued that root pressure cannot be the prime operative force when sap is ascending most rapidly.

We must note again, however, that White (see p. 333) has reported that in his experiments on growing excised roots *in vitro* he measured root pressures that increased daily to magnitudes probably greater than 10 atmospheres. The bearing of these experiments on problems of the ascent of sap has not yet been considered by authorities on this subject. White has suggested that root pressure may be important under conditions of low transpiration, *e.g.*, in tropical forests during the rainy season.

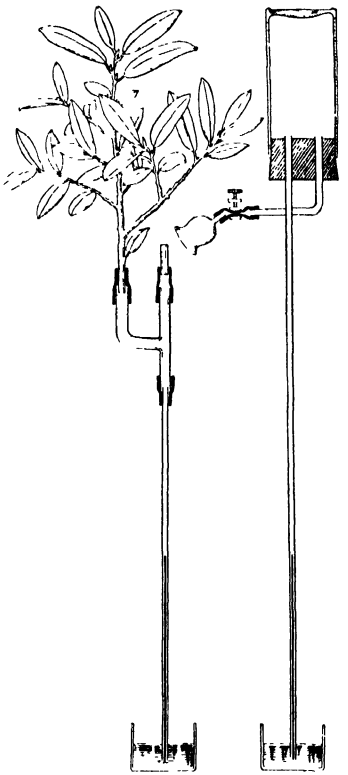
The exertion of the motive power as a property of the shoot system. Having eliminated the possibility that pump action from below, or at different levels along the conducting channels, causes the ascent of sap to the tops of tall trees, physiologists then considered those forces which may be operative in transpiring shoots. The preliminary work justified the formulation of a tentative hypothesis, viz., that tensile pulls exerted in the transpiring shoot may provide the motive power that propels sap, and led to the development of the cohesion theory of Dixon and Joly.

C. The Cohesion Theory of the Ascent of Sap

The pulls occasioned by transpiration, and caused by imbibition or osmotic suction. Parallel experiments (figs. 14 and 15) indicate that evaporation from the wet walls of a porous pot, and transpiration by the living cells of a leafy shoot, may each give rise to pulling forces on continuous columns of liquid, and thereby actuate an upward movement. Considering the physical model (fig. 15) first, we note that a " tendency to a vacuum " will not alone account for the ascent of liquid, seeing that the mercury has, in certain experiments, been pulled up to heights greater than 76 cms. A reasonable explanation may be offered if we suppose that there are minute capillaries within the walls of porous pots. In such a system a relatively long continuous column of liquid can be supported : for it is well known that the height to which a given liquid rises in a capillary tube of given material is inversely proportional to the diameter. For example, water rises 3 cms. in a glass capillary tube of 1 mm. bore, 30 cms. when the bore is 0·1 mm., 300 cms. when the bore is 0·01 mm., etc. The entry of water into the minute capillaries of a dry pot actuates an upward movement in the whole liquid column within the pot and glass tube and causes a slight rise. Owing to the

powerful forces of adhesion between water and earthenware, water and glass, and water and mercury, and of cohesion between the molecules of water, the liquid column keeps entire.[1] When the water arrives at the outer surface of the pot, evaporation occurs from the concave menisci of the narrow water columns in the capillaries. As long as the liquid column below remains entire, continuous evaporation occasions a continuous mass-movement of water towards the capillaries in the pot. In brief, we have a dynamic system open to the atmosphere in which a pulling force results from the high forces of adhesion between water and porous pot, and causes the upward movement of liquid.

According to the theory of Dixon and Joly analogous events may occur in the system with the leafy shoot (fig. 14) when this is placed in an *unsaturated atmosphere*. They imagined that capillaries approaching molecular dimensions traversed the wet walls of transpiring cells, and pointed out that the diameters of such capillaries would be small enough to support columns of water of heights far greater than those of the tallest trees. The first stage in transpiration would then be the absorption of heat energy and the evaporation of water from the concave menisci of the exceedingly narrow water columns in the capillaries, and the second, diffusion into the outer atmosphere. Once more, therefore, we have a dynamic system open to the atmosphere. In this system evaporation leads to the development of unsatisfied



FIGS. 14 and 15. Experimental systems for demonstrating that both transpiration and the evaporation of water from a porous pot may set up pulling forces which cause liquids (*e.g.*, water and mercury) to ascend in vertical glass tubes.

[1] The water hammer (fig. 16) may be used to demonstrate the existence of forces of adhesion and cohesion. Thus by carefully tilting the water hammer from position A to position B and finally to position C, it can be shown that the forces of adhesion between water and glass, and cohesion between water molecules, may be sufficient to resist the action of the gravitational force on the whole column of water.

imbibitional forces. These occasion tensions on the solutions in the turgid transpiring cells of the leaf, which are transmitted to the liquid in the conducting channels of the xylem, and thence to the continuous column of liquid (water and mercury) in the glass tube below.

In *saturated atmospheres* transpiration ceases; but, according to Dixon, secretory activity of living cells, promoted by respiratory energy, may initiate pulls. In 1897 he observed that eosin solutions rose in cut lower ends of branches of certain plants, whose leafy shoots were either enclosed in a saturated space or even completely submerged in water. Smith, Dustman, and Shull (*274*), in similar experiments failed to obtain rises of eosin when water deficits in the experimental branches had previously been satisfied. Dixon (*76*), however, has made further experiments and maintains his original contentions. For example, he observed moderate rises of eosin, even at low temperatures (*e.g.*, 9·5° C.) and in rather a weak specimen of *Chrysanthemum sinense* that had previously been totally submerged in aerated water for twenty-four hours. Aeration is important to provide oxygen for respiration; sometimes the supply may be assured by illuminating submerged green shoots.

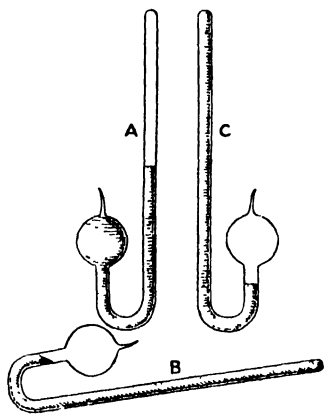


FIG. 16. The water hammer (From Dixon, *75*; see footnote on p. 131.)

Dixon and Joly maintained that in a whole plant rooted in the soil, tensions are transmitted by the continuous columns of liquid in the xylem all the way down to the level of the absorbing region of the root. At this level, lateral movement of water under tension may occur from the soil solution, and induce an upward movement of water in the soil from the water table. The continued movement of water in soil and plant depends upon the existence in the shoot system of a pulling force occasioned by transpiration (hence the term transpiration stream), and on the cohesive properties of sap (see next sub-section). The forces of adhesion between water and cell wall material, which are operative in the capillaries of cell walls tending to dry during transpiration, are sufficiently high to account for the ascent of sap to the tops of the tallest trees. Other tractions are also effective. In wilting tissue positive suction pressures develop and increase, and water is drawn from tracheæ until new equilibria are established between the tensions existing in the tracheæ and the turgor pressures in the adjacent cells. In shoot apices, unfolding

buds (see Priestley, *233*), differentiating secondary tissues, etc., forces of imbibition and osmotic suction will draw water in the direction of newly formed and enlarging cells.

Several phenomena may be explained by supposing that distributed pulls of different magnitude are simultaneously operative on the continuous columns of sap. For instance, competition between the transpiration pull and the suctional pull in growing regions would explain the fact that the rates of growth and transpiration are often inversely related. An interesting illustration of competition between distributed transpiration pulls has been recorded. Yapp (see Maximov, *196*) observed that during a period of drought the shaded leaves of a weeping ash wilted every day while those directly exposed to the sun's rays remained perfectly fresh. We may suppose that the intense transpiration pulls exerted by the leaves that were exposed to the sun deflected water from the conducting channels on the shaded side of the tree.

The tensile strength of sap and its taxation. Dixon and Joly attributed the upward movement of sap at the rate of the transpiration stream to the tensile forces exerted by the ultimate parts of the shoot system on the continuous column of liquid below. They suggested that a column of sap is comparable with a steel wire, in that sap as well as wire may be stretched by tensile forces. To test their hypothesis they performed experiments to determine what tensions would be necessary to pull the sap up at the requisite rate, and whether the forces of cohesion and adhesion operative in the sap were sufficiently high to prevent the breaking of columns of liquid in the conducting channels. In short, they set out to determine what taxation is imposed on the tensile strength of sap, and the sap's capacity to meet this taxation.

It is clear that a tension of 10 atmospheres would be required to hold in position a vertical 340-ft. column of water. In a 340-ft. tree, not only must the sap be held in a continuous column in the xylem, but it must move at a sufficient rate to make good the water transpired. The frictional resistance of the walls in the conducting tracts tends to retard movement. Consequently, in order to overcome this resistance, greater tensions than 10 atmospheres must be exerted in the transpiring regions of a 340-ft. tree. Dixon found that sap moved at the rate of the transpiration stream in a piece of horizontally placed yew stem, of length L feet, when propelled by a pressure head of water equal to the length of the piece of stem used, *i.e.*, a pressure of L/34 atmospheres was required to overcome the frictional resistance in the conducting channels. Clearly, in order to propel water at the same rate against the gravitational force in a piece of stem placed in a vertical position a pressure of 2L/34 atmospheres would have to be applied. Since the frictional resistance

offered by wood to the passage of water varies from plant to plant (*cf.* specific conductivities which are the inverse of specific frictional resistances), we must not suppose that the relation, tension required = 2L/34 atmospheres, is applicable to all stems. But assuming this relation to hold for a certain 340-ft. tree, we should conclude that a tension of 20 atmospheres would have to be exerted to effect the ascent of sap at the rate of the transpiration stream. We have taken a very tall tree as an example : 10 atmospheres would suffice for the tallest British trees. It is, therefore, a significant fact that osmotic pressures of the order of 10–20 atmospheres have been measured in the leaves of trees. These would become operative in drawing water from tracheæ into the cells of wilting leaves. In turgid shoots the living cells possess high turgor pressures. These enable such cells to withstand without crumpling the high tensions that may be transmitted through them to the tracheæ from the evaporating surfaces of transpiring tissues. Inasmuch as the forces of adhesion between water and cell-wall materials may be well over 1,000 atmospheres, we know of the existence of tensile forces in transpiring regions that are more than sufficient to actuate the ascent of sap at the requisite rate.

The critical question now arises, can sap withstand tensions greater than 20 atmospheres ? In other words, are the forces of cohesion between like and of adhesion between unlike molecules of sap, and of adhesion between molecules of sap and the lignified walls, sufficient to maintain continuous upward moving columns of liquid when these are subjected to pulls greater than 20 atmospheres ?

Dixon and Joly extended the physical experiments carried out last century by Berthelot. They placed a volume (v_1 ccs say) of air-free water in a thick-walled capillary tube at t_1° C. The water was heated to t_2° C., at which temperature it completely filled the tube. Let us suppose it then occupied v_2 ccs. The tube was sealed and cooled to t_1° C. It was found that the water still filled the tube ; *i.e.*, water which had initially occupied v_1 ccs at t_1° C. was occupying in a state of tension v_2 ccs at the same temperature. In order to find the magnitude of this tension, Dixon and Joly calculated the pressure required to bring about a diminution in the volume of water from v_2 ccs to v_1 ccs at t_1° C. In their experiments they used different lengths of tube and different initial volumes of water, and their results indicated that the stretched water must often have been under tensions far greater than 100 atmospheres, *i.e.*, the forces of adhesion between water and glass, and cohesion between water molecules, proved high enough to maintain a continuous column of liquid under tensions greater than 100 atmospheres. Dixon performed experiments with plant sap also, and obtained remarkable results. He found that sap extracted by centrifuging pieces of a branch of *Ilex aquifolium* withstood a tension of 207 atmospheres. It should be

CHAPTER IX

THE CONDUCTION OF SOLUTES

A. The Conduction of Solutes Across Parenchymatous Tissues

WERE the conduction of solutes in parenchyma purely a physical process one would expect the rates of conduction, for a given solute, to be governed by diffusion gradients and the permeabilities of membranes. The migration of the ions absorbed from the soil has already been considered from this standpoint. Other important sources of diffusible solute molecules (*i.e.*, solutes in crystalloid solution) are green cells in the light, and storage cells in which reserve foods are being hydrolyzed. The production of a diffusible solute at these sources would steepen a pre-existing diffusion gradient for that solute, and so promote conduction. Diffusion gradients may also be steepened by the removal of the diffusing solutes from the medium of diffusion (*cf.* p. 92), as happens in the consumption of migratory solutes in growth and respiration, and in the formation of storage products. Upon such removal solutes will continue to diffuse towards the regions where they are consumed or stored, until supply fails at the source.

One would expect that the slowing down of processes concerned with removal would lead to a reduction in the rate of conduction, and that more rapid removal would promote conduction. Experimental evidence supports this view. For example, Puriewitsch excised the embryo from a grain of maize planted in the soil, and observed that the removal of carbohydrate from the endosperm was inhibited by this operation. When the endosperm was placed on the point of a little cone of plaster of Paris dipping into water, *i.e.*, upon substituting an artificial sink for the natural one, viz., the embryo, the carbohydrates migrated from the endosperm through the plaster of Paris into the water.

In growing plants production and metabolic removal work together in maintaining diffusion gradients. In a growing potato plant the sugars, and other diffusible solutes (*e.g.*, amino acids and amides) that accumulate in green leaves as a result of photosynthesis and subsequent changes, continually migrate to the growing regions, to the storage cells of the enlarging tuber, and to other tissues where metabolism is in progress. When potato tubers sprout, food reserves are mobilized, and migrate as diffusible sugars, amino acids, etc., to the metabolizing cells of the developing shoots. So also for germinating seeds, and at all stages during the growth of herbs, shrubs, and trees, the migration of solutes is governed by metabolic events at

source and sink. Thus greater activity at a sink means more rapid removal from a source. An observation of Mason and Phillis (*193*) illustrates this point. Cotton plants were grown in water cultures containing different amounts of nitrogen. Growth, which is a sign of utilization of food at sinks, was most active in those plants which received most nitrogen; and it was observed that their leaves contained little or no starch at dawn each day. On the other hand starch was usually present at dawn in the leaves of plants poorly supplied with nitrogen; the nightly activity at the various sinks had not been sufficient to deplete the leaves of foods stored during the day.

We do not yet know what processes other than physical diffusion in a continuous liquid system comprosed of wet cell walls, protoplasts, and vacuolar sap, play a part in the conduction of solutes in parenchymatous tissue. The physical diffusion of solutes is an extremely slow process. Thus it has been estimated that in an aqueous system in which a ten per cent. solution of sodium chloride, a salt possessing high mobility, is continuous with pure water, it would take nearly a year for 1 mg. of the salt to travel 1 metre by diffusion. It would appear that in living tissue there must be some protoplasmic mechanism for promoting along the directing concentration gradients the conduction of solutes that diffuse in water even more slowly than does sodium chloride. Moreover, cell walls and protoplasmic membranes resist the passage of diffusible substances (Steward, *284*). It has been suggested that streaming movements in living cells may, by mechanically mixing solutes, promote migration, and that migration may take place from cell to cell along the minute canals enclosed by the protoplasmic threads, which often penetrate the cell walls of parenchymatous tissue. And it must be borne in mind that solutes may be secreted from cell to cell, energy for the process coming from respiration. But the question of what mechanism, if any, promotes translocation across living tissue is far from being settled. There is, however, abundant evidence that rapid longitudinal transference of solutes is effected in the specially differentiated conducting elements of the xylem and phloem.

B. The Conduction of Solutes in the Xylem

The sap that is conducted in the xylem is not pure water but a solution of mineral salts and metabolic products. The solute particles are passively carried in any mass movement of the sap that may occur under the agency of transpiration pulls, osmotic and imbibitional suctions, or root pressure. One may assume that the rate of migration of solutes would be that of the transpiration stream, which, as we have seen, may be considerable. There are not wanting supporters for the view that transpiration serves the useful or even essential function of promoting the conduction of solutes in the xylem (p. 111).

Dixon and Atkins analysed the sap obtained by centrifuging tracheæ that had been removed from the branches and roots of a number of trees, and found that in addition to inorganic salts, sugars, particularly cane sugar, were present, and usually in higher concentrations than the inorganic salts. They came to the conclusion that the starch in the sheath of wood parenchyma round the vessels is the proximate source of the carbohydrates that pass into the transpiration stream. Fisher had earlier detected reducing sugars in wood vessels, and proteins, amino acids, and amides, in sap issuing under pressure during bleeding. More recently the presence in the transpiration stream of organic as well as inorganic solutes has been demonstrated by Priestley for the vine and by Anderssen for the pear tree.

Since the middle of last century Malpighi's ringing experiments (p. 126) have been used as a pattern in many systematic investigations of the translocation of solutes. Hartig's experiments, reported in 1858, appear to have been the first in the opening phase of renewed enquiry into the subject of the physiology of conduction. He removed a girdle of bark from a stem of a woody sapling in the autumn after starch had been stored in the basal part of the plant. He found that in the following spring all the starch, which had been held in the winter months as solid grains in the xylem parenchyma and medullary rays, disappeared from the basal part (*i.e.*, from below the girdle). He suggested that starch was changed into soluble products which migrated to the vessels and tracheides, and were carried upwards in the ascending sap. Curtis has, however, offered an alternative explanation, viz., that foods stored below the girdle might have been used in local growth, or have travelled downwards in the phloem to the roots (section C).

The functional significance attaching to the presence of inorganic and organic solute molecules in tracheal sap has been the subject of much debate. It appears that the concentration of sugars is relatively high in the tracheal sap of woody perennials in the spring, but falls when transpiration becomes active after the unfolding of the young leaves. For the concentration of sugars Atkins (7) has reported that " the vernal maximum coincides with the period of greatest root pressure, and is simultaneous with or just prior to the opening of the leaf buds." It is difficult to resist coming to the conclusion that those solutes which are rapidly carried in the rich tracheal sap in the spring are of great nutritional value to the developing buds. Doubtless in the summer, organic solutes, although then in much greater dilution, are still passively carried in the transpiration stream, and some will be consumed and some stored. But it has long been held, and recent work substantiates the view (section C), that it is nutritive sap from green leaves which is chiefly used in the summer, and that this is conducted in the sieve tubes. In 1923 Dixon criticized this view. He measured the rate of accumulation of carbohydrates in a potato tuber, and the cross-sectional area of the phloem, and calculated that conduction of carbohydrates in the sieve tubes would necessitate a rate of migration of solute particles as high as 50 cms. per hour. He pointed out that there is no evidence that mass movements of liquid occur in sieve tubes, and concluded that these tissue elements could not therefore be the channels for so rapid a migration of carbohydrates. He suggested that only by the passive carriage of solute particles in the sap moving under tensile forces in the xylem could such high rates be attained, and performed experiments with dyes to show that solutes may be carried in the transpiration stream downwards as well as upwards.

Whereas Dixon, for a period, attached supreme nutritional importance to the solute molecules in tracheal sap, not only in the spring, but at all

times in the growing season, other workers have held that the nutritional significance of solutes in tracheal sap is negligible. Curtis (see 66) asserted that even mineral salts (*e.g.*, nitrates) can be conducted upwards by way of the tissues external to the wood at a sufficient rate to allow normal growth. But Mason and Maskell found in their experiments on the cotton plant that the amount of inorganic nitrogenous compounds in the stem and leaves above that part of the stem from which a girdle of bark had been removed continued to increase after the girdling operation. This finding, although not disproving Curtis's assertion, provided substantial evidence in support of the widely and long held view that, throughout the growing season, inorganic salts absorbed from the soil are normally carried to all parts of the plant in the transpiration stream.

C. The Conduction of Solutes in the Phloem

In this section we must note that evidence exists of the conduction in the phloem of salts absorbed from the soil, and then proceed to consider a subject that has attracted far more attention, viz., the probable function of phloem in the translocation to all parts of a plant of foods manufactured in green leaves.

Possible movement of absorbed salts in the phloem. Several workers have considered the possibility that mineral salts absorbed from the soil may under certain conditions move upwards in the phloem as well as in the xylem. Using a novel and interesting method Gustafson and Darken (*107*) obtained results which support Curtis's evidence of such a movement. They traced by means of an electroscope the upward movement in the bark of certain cuttings, of radio-active potassium dihydrogen phosphate, which had been prepared from red phosphorus irradiated in a cyclotron apparatus, and concluded from their results that movement is just as rapid in the phloem as in the xylem. Measures were taken to prevent leakage of solutes from the xylem into the tested portions of bark.

Sieve tubes as the channels for transport of foods from leaves. By the middle of last century the extension of knowledge of plant anatomy had considerably increased the scope of ringing experiments.[1] Thus Hartig had in 1837 described sieve tubes and their contents, and it was known that the arrangement of vascular bundles and the distribution of tissue elements varied from plant to plant. Hanstein (1860) found that root formation was inhibited by the removal of extracambial tissues from dicotyledonous plants with a single ring of collateral bundles. Girdling the stems of monocotyledonous plants, which, of course, possessed scattered bundles, or those of certain dicotyledonous plants that possessed an inner ring of bundles or a single ring of bicollateral bundles, had no such inhibitory effect. It was concluded from these experiments, in which growth was used as an index of translocation, that, in the absence of phloem,

[1] See Jost (*158*), and the article by Mangham (*187*) for fuller accounts of the experiments made in the last century.

nutritive sap could not be conducted at a sufficient rate for root formation, but whenever phloem was present this necessary rate was attained. Hanstein assigned to the sieve tubes the function of conducting the necessary food stuffs from green leaves or other sources of supply. The same inference has been drawn from the results of nearly all the later work on the conduction of solutes.

In his experiments Hartig (1858) used the appearance or disappearance of starch as an index of the migration of carbohydrates, and reported that starch accumulated above, but not below, a ring cut in the stem of a leafy shoot. He inferred that wood cannot carry products of assimilation downwards (*cf.* his conclusions concerning the upward conduction of foods, p. 189). He also found that when he left a narrow vertical strip of bark starch formed at its base, while a bridge of step form did not allow downward migration to occur sufficiently rapidly for starch formation. He concluded that conduction in the bark takes place only in a longitudinal direction. Sieve tubes have long been regarded as well adapted for this purpose.

The results of experiments on green leaves have, in general, substantiated Hartig's and Hanstein's conclusions. Sachs found that starch disappeared in the dark from leaves attached to shoots more rapidly than from single detached leaves, and inferred that translocation of carbohydrates as well as respiratory oxidation had occurred in the experiments with attached leaves. Later workers have operated on petioles of attached leaves to ascertain the effects of removing various tissues on the rate of depletion. Czapek, for example, selected for experiment plants with petioles which possessed different anatomical structures. When the vascular bundles ran separately through the petioles (*e.g.*, Vitis), he found that by making incisions into the petioles so as to remove the vascular tissue he could almost completely prevent the removal of starch from that part of the leaf above the incision. Starch disappeared rapidly from the other side of the leaf. The operation was performed so as to maintain connection, by parenchymatous tissue, between the leaf blade and stem on the cut side of the petiole. Czapek inferred that the migration of carbohydrates through parenchyma was not sufficiently rapid to account for the removal of carbohydrates along the intact part of the petiole. His experimental results also lent support to Hartig's view that the deflection of migrating solutes through parenchyma to intact elongated tissue elements in the vascular bundles occurs to but a negligible extent. When, however, he made incisions in petioles, in which there was anastomosing of bundles (*e.g.*, Lady fern), or cross-connecting of sieve tubes (*e.g.*, Gourd), deflection of the migrating carbohydrate molecules must have occurred, for that part of the blade above the incision lost starch at about the same rate as the other part. Czapek concluded that the essential structural

requirement for the conduction of carbohydrates is a continuous system of sieve tubes. It must be remembered, however, that he never succeeded in removing the parenchymatous bundle sheath. Schimper had earlier suggested that this sheath might function in the conduction of carbohydrates (see Mangham's experiment, below). Nevertheless, general support was given at the beginning of the present century to Czapek's hypothesis that starch in the mesophyll undergoes hydrolysis to sugar, which then migrates by way of the bundle sheaths into the phloem. Although there had been no visual demonstration of this migration, it was known that more than half the dry weight of sieve tubes may consist of sugars. Mangham applied microchemical tests in order to follow the actual movements of sugars in leaves. He demonstrated the presence of cane sugar in the sieve tubes, and, by detecting the emptying of this sugar from the parenchyma of the bundle sheath into the sieve tubes of the small veins of leaves, refuted Schimper's hypothesis, and provided strong support for Czapek's view.

It has long been known that the slimy contents of sieve tubes are proteinaceous, and weight has been attached to this and certain other facts (*e.g.*, the free passage that is provided for solutes in colloidal dispersion by the pores of sieve plates) by those who have supposed that nitrogenous organic substances and carbohydrates migrate together. Indeed, until Dixon made his provocative suggestion (p. 139), the hypothesis that all foodstuffs move "down the bast" was not seriously challenged. Curtis had already begun his important researches,[1] and soon entered the lists in opposition to Dixon. His work and that of Mason and Maskell (*190*) very quickly left defenders of the view that food is conducted in the sieve tubes, in renewed possession of the field.

In the last century the development first of plant anatomy and then of microchemistry promoted researches on conduction. In the present century quantitative methods for estimating carbohydrates and nitrogenous substances have been considerably improved, and are being increasingly used in attempts to elucidate physiological problems. It must be realized, however, that these methods are as yet by no means perfect. Particularly may this be said about the methods of estimating nitrogenous substances. Nevertheless, the quantitative experiments of Mason and Maskell have already defined many new problems for detailed enquiry, in addition to confirming the general conclusions arrived at by Czapek and others from the results of cruder experiments. Mason and Maskell determined the

[1] Curtis (*66*) has written summarized accounts of his and other researches on translocation in plants. In this monograph he surveys critically the experimental evidence and theories concerning the paths and the methods of movement of solutes.

changes which occurred in the amounts of various diffusible carbohydrates and nitrogenous substances (which term they shorten to nitrogen) in leaves, wood, and bark (*i.e.*, tissues external to the wood), when plants of a selected strain of Sea Island cotton were placed

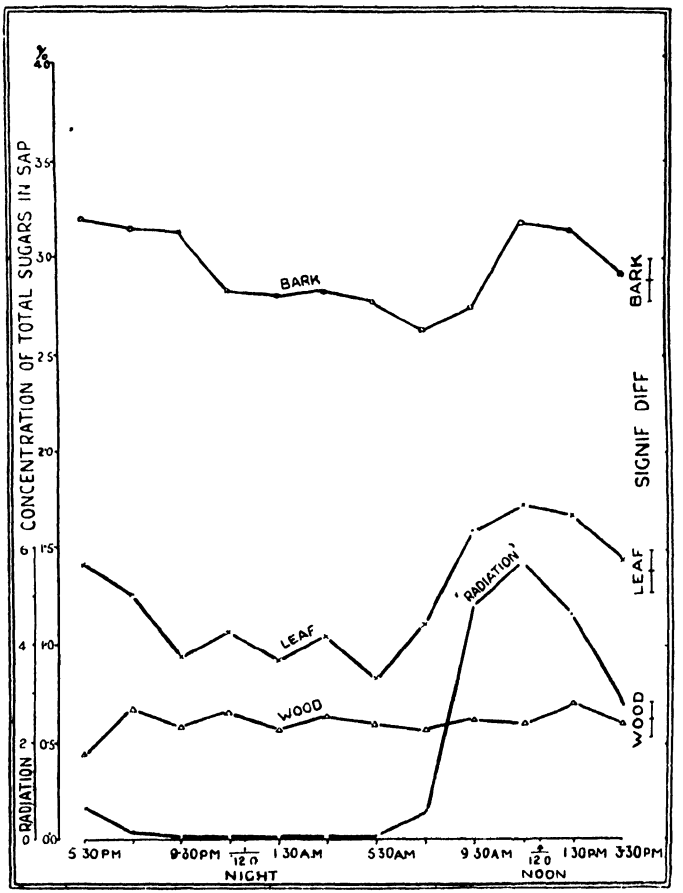


FIG. 17. Diurnal variations in sugars expressed as grams of total sugars in 100 ccs of sap of the leaf, bark, and wood of the cotton plant. (From Mason and Maskell, *190.*) Notice that the variations in the leaf and bark exceed the significant differences for these parts, while the variations in the wood are not significant.

under diverse experimental conditions. They subjected their experimental results to statistical analysis, and, after making proper allowance for sampling and experimental errors, drew conclusions only when the numerical differences observed after changing the experimental conditions were greater than those which might have

resulted from pure chance. The results indicated that "the gross phenomena of nitrogen transport show a striking similarity to phenomena of carbohydrate transport." For the sake of brevity, therefore, we shall here consider nitrogen and carbohydrate transport together.

It was found that diurnal variations in total sugars and nitrogen in the green leaf led, after a lag period, to significant variations in their concentration in the bark; the concentrations in the wood, however, remained constant (see figs. 17 and 18). Plainly these facts could be explained by supposing the bark (cortex plus phloem), and not the wood, to be the channel of translocation. Moreover, fluctuations in the concentrations of carbohydrates (particularly cane sugar)

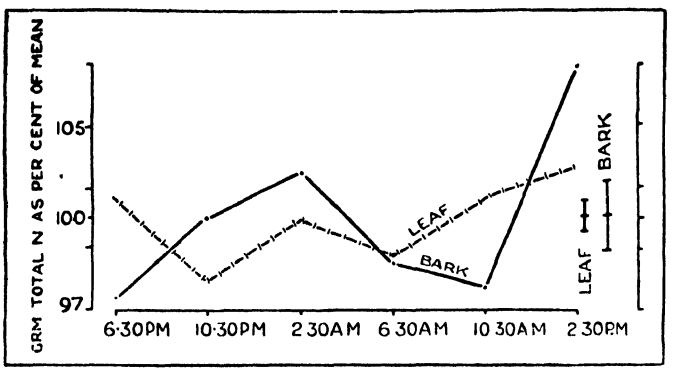


FIG. 18. Diurnal variations in the total nitrogen content of the leaves and bark of the cotton plant. Results expressed per 100 gm. residual dry-weight. Standard deviation due to sampling shown by vertical lines on the right. (From Mason and Maskell, *191.*)

in the sieve tubes corresponded with those occurring in the bark, and much weight was attached to the fact that from zone to zone a high positive correlation was displayed between the number of sieve tubes and the concentration of cane sugar. Suggestive results of a similar kind were obtained for *residual nitrogen* (see below), and the general conclusion arrived at was that carbohydrates and nitrogen migrate in the bark, and particularly in the sieve tubes.

Ringing experiments added weighty evidence in favour of the view that downward migration occurs in the bark and not in the wood. Complete ringing of a stem below a foliage region caused carbohydrates and nitrogenous organic compounds to accumulate above the ring in the bark, wood, and leaves, and interrupted the flow below the ring. It was inferred that the wood alone could not conduct the descending nutritive sap. Nevertheless, Mason and Maskell, on repeating Dixon's experiment (p. 189), found that dyes moved across

the ringed portion of stem. Hence it appears that, although foodstuffs travel in the phloem, certain solutes might, on occasions, pass out of leaves *viâ* the xylem. By showing that transport of organic solutes occurred at nearly the normal rate after a ring of paraffined paper had been inserted between bark and wood of an unringed stem, Mason and Maskell established the fact that contact between wood and bark is not essential for normal conduction. Having previously demonstrated that wood does not conduct they concluded that continuity of the tissue elements in the bark is the sole structural necessity for the downward flow of dissolved foods. They confirmed this conclusion by demonstrating the passage of solutes into levered-up flaps of bark.

Mason and Maskell found that the effects induced by removing developing flower buds or bolls (the fruit of the cotton plant) were the same as those induced by isolating the root system through ringing the stem, viz., foodstuffs accumulated in the bark of the rest of the plant, and noteworthy increases occurred in the amounts of sugar (mostly of *cane sugar*) and residual nitrogen in the sieve tubes. It was therefore concluded that these tissue elements also serve as the channels for the upward conduction of foodstuffs from the foliage leaves to the flower buds and bolls, where they are converted into the substances present in the developing parts of the cotton seed.

Direction and rate of movement of solutes. Mason and Maskell concluded from their results that longitudinal conduction of mobile carbohydrates from green leaves towards roots, or developing flower buds and bolls, takes place in the sieve tubes along a positive dynamic concentration gradient, *i.e.*, from regions of ever-changing higher to regions of ever-changing lower concentration. Consequently they inferred that translocation is effected by a *process analogous to diffusion* (see below).

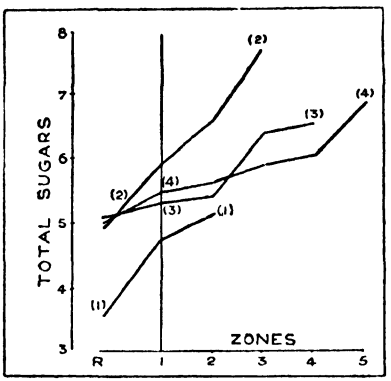
There can be no doubt that hexose sugars are mobile substances, but Mason and Maskell found that, in the cotton plant, carbohydrate migrates in sieve tubes pre-eminently in the form of cane sugar. They suggested that hexose sugars diffuse along a concentration gradient towards the sieve tubes, where a considerable fraction is changed to cane sugar. Possibly companion cells assist in this synthesis. A considerable head of sugars (mainly cane sugar) would thus be set up in the sieve tubes. The simultaneous production of hexoses in the mesophyll (either as a result of photosynthesis or through the hydrolysis of higher carbohydrates) and the synthesis to cane sugar in the sieve tubes, would preserve concentration gradients for hexoses f. esophyll to sieve tubes, and for cane sugar away from the head in the sieve tubes. Since leaf cells are impermeable to cane sugar, carbohydrate cannot leak back as cane sugar into the mesophyll.

Mason and Maskell found that during development and while sugars were diffusing away from green leaves an acid-hydrolyzable polysaccharide was stored in the bark and in the wood. Its concentration in the bark increased from the upper regions of the stem downwards to the root. This negative gradient steepened as development proceeded. Since sugars are easily estimated independently of polysaccharides, a negative gradient for such a storage carbohydrate does not obscure a positive gradient for mobile carbohydrates, viz., sugars.

It is not so easy to distinguish between storage and mobile components of nitrogenous substances. Total nitrogen, protein nitrogen, and crystalloid nitrogen fractions, have been quantitatively estimated in this research. Crystalloid nitrogen has been split up into amino-acid, amide (mainly asparagine), ammonium and nitrate fractions; leaving a considerable residue unaccounted for, which is described as *residual nitrogen*. Mason and Maskell have not yet determined beyond doubt what fraction or fractions of the nitrogenous substances in the bark represent mobile nitrogen. They tacitly admit that what cannot be defined cannot be estimated. Nevertheless, they have collected much valuable quantitative data, and have made certain noteworthy tentative suggestions. They found that the concentration of total nitrogen increased from the upper regions downwards to the root, and the results of more recent work (Mason and Phillis, *193*) showed that this negative gradient exists during development independently of the nitrogen supply to the roots. Nitrogen starvation did not reverse the gradient. Clearly, therefore, a diffusive migration of nitrogen, longitudinally downwards, is not determined by the existence of positive gradients for total nitrogen. Although there is a protein component of this total nitrogen, Mason and Maskell found that negative gradients mainly result from changes in the concentration of crystalloid nitrogen. They assert however, and this is a crucial point in their argument, that these negative gradients for total nitrogen (and, therefore, for crystalloid nitrogen also) are compounded of a positive dynamic gradient of mobile nitrogen, and a steeper negative gradient of storage products containing nitrogen. The latter, they state, has no concern with the diffusive migration of nitrogen. As a result of this earlier work they concluded that, in the cotton plant, asparagine in crystalloid solution is the main constituent of such storage products as mask the positive component for mobile material. Moreover they found positive gradients in the bark for residual nitrogen, and inferred that this fraction contains mobile material. Movement of amino compounds, ammonium salts, and nitrates, was not excluded.

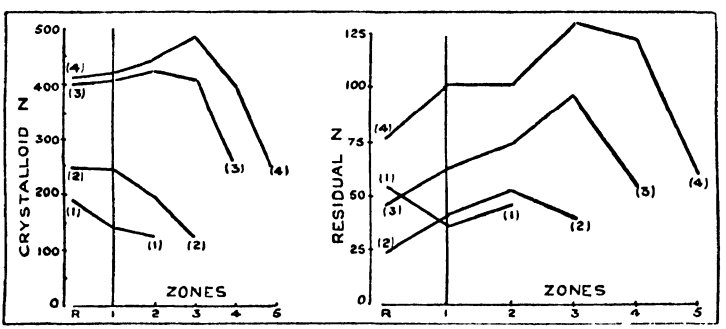
In a later contribution they summarize evidence in favour of their views. They maintain (*a*) that when longitudinal movement of

carbohydrate and nitrogen was stopped, the original positive vertical gradient was steepened, and (*b*) that the reversal of the normal direction of the movement of materials along the main axis was accompanied by a reversal of the gradient of sugar concentration in the inner region of the bark, and the original negative gradient of crystalloid nitrogen was steepened. They attributed this steepening to the persistence of the negative gradient for the storage component of crystalloid nitrogen, accentuated by the abolition or reversal by



(*a*)

FIG. 19. Concentrations in the bark of the cotton plant of total sugars (fig. 19, *a*), crystalloid nitrogen (mainly asparagine) (fig. 19, *b*), and residual nitrogen (fig. 19, *c*), expressed in mgs per 100 gm water in the plant, in root and successive stem zones, (1) 70 days, (2) 98 days (3) 131 days, and (4) 159 days, from sowing seed. Note the positive vertical gradients for sugars at all collections, and that these were steeper at the earlier collections when growth was more rapid. Variation in cane sugar concentrations was mainly responsible for these positive gradients. Note also the general tendency from above downwards towards a negative gradient of total crystalloid nitrogen, indicating the presence of the storage component (asparagine), and, in the lower zones, towards a positive gradient for residual nitrogen, suggesting that at all stages this fraction contained the mobile nitrogen compounds. From Mason and Maskell (*192*).



(*b*) (*c*)

experiment of the positive gradient for the mobile component. Since there was no masking storage component among the sugars (the storage carbohydrate being a polysaccharide) experiments led to the abolition or reversal of gradients for sugars as a whole.

Moreover they submitted new and cogent evidence in favour of their diffusion hypothesis. Analyses were made from time to time during the growth of the cotton plant, whilst it was still in the vegetative state, *i.e.*, before flowering began. The concentration gradients for total sugar were consistently positive (fig. 19, *a*). Cane

sugar concentrations primarily determined the gradients, but those of hexoses also contributed especially from the base of the shoot to the root. The gradients were steeper at early stages when growth was most vigorous. During vegetative development crystalloid nitrogen accumulated in storage products (see p. 179) in the bark, reaching a maximum concentration after about four months from sowing the cotton seed. At all stages gradients were negative from the upper regions of the stem to the roots (fig. 19, *b*). It was found that asparagine was mainly responsible for accumulation and for the existence of the negative gradients. Residual nitrogen showed positive gradients at all stages of development (fig. 19, *c*), providing further evidence in favour of the diffusion hypothesis for mobile nitrogen.

Evidence consistent with the diffusion hypothesis is the reported existence in the phloem of positive gradients for cane sugar and for residual nitrogen from leaves to developing cotton bolls, which act as sinks because of their anabolism and respiration. Mason and Phillis (*193*) obtained further supporting evidence that positive gradients for mobile nitrogen are masked by negative gradients for storage nitrogen when they found that with flowering and the development of bolls, nitrogen was withdrawn from leaves and bark. The original negative gradient in the bark was reversed, because there was a relatively greater depletion of nitrogen from the lower regions, where, it was suggested, nitrogen is stored during vegetative development in greater concentrations than in the upper regions. The existence of positive gradients for mobile nitrogen became manifest after the translocation of storage crystalloid nitrogen to the flowers and bolls.

Mason and Maskell (see *e.g.*, *192*) have also obtained and, in similar fashion, examined evidence of movement in the bark of substances containing potassium, phosphorus, and calcium. They consistently found positive gradients for potassium, but in the earlier stages of development phosphorus showed a negative gradient in the bark (and also in the wood). They attributed this negative gradient to the presence of a storage component (*cf.* nitrogen). The general conclusion drawn was that the downward movement, from the foliage regions, of each class of substance, is determined by the existence in the phloem of positive concentration gradients from sources to sinks for the mobile forms within each class, and not for the class as a whole. Also they favour the working hypothesis that mobile substances from different classes move independently in the phloem by a process analogous to diffusion.

Now the observed rates of migration of food substances are much too rapid to be accounted for by simple physical diffusion even along very steep diffusion gradients. Mason and Maskell estimated that

the observed diffusion constant for cane sugar is forty thousand times greater than the diffusion constant for two per cent. cane sugar in water at 25° C. The maximum concentration of cane sugar in the sieve tubes would rarely be as great as this. Hence they insist that longitudinal migration is by a process *analogous to diffusion*, and not by simple physical diffusion. It is analogous to diffusion in that the rate as well as the direction of migration is controlled by concentration gradients. For example, it was observed that variations in the sugar gradients and the rates of transport of sugar from the bark to the bolls were significantly correlated. In one experiment the rate by day, when through photosynthesis the head of sugar in the green leaves would be relatively high, was 4·5 times greater than by night.

The weakness of the diffusion hypothesis, as Mason and Maskell admit, is that no one has yet apprehended what mechanism in the plant actually accelerates diffusion (*i.e.*, enhances the value of the diffusion constant for a given solute when dissolved in the sap of sieve tubes) and so promotes diffusion along concentration gradients. It should be noted that streaming of protoplasm cannot afford a general explanation, as it has but rarely been observed in mature sieve tubes. But it has long been known that the sap in sieve tubes exerts turgor pressure on the walls, and may exude under pressure when a stem is cut. The recognition of this fact led to the enunciation of the " mass-flow hypothesis," for which in recent years Münsch has been the most noted advocate. For a considerable discussion see Curtis (*66*). The central idea in this attractive hypothesis is that the dissolved particles are passively carried (*cf.* the migration of particles in the xylem) in the sap, which moves as a whole in the vacuoles of sieve tubes and through the pores of sieve plates from regions of higher to regions of lower turgor pressure. It has been suggested that the lower turgor pressures result from the withdrawal of water from the sieve tubes by the tracheæ, and the high turgor pressures from the reverse movement, *i.e.*, flow in the phloem is connected with that in the xylem. Dixon (*76*) has recently reported that by tracing the convection of heat in stems, he has obtained " clear evidence that, while the transpiration stream is moving upwards, there is often another stream which is moving simultaneously downwards." He suggests that this may be mass flow in the phloem, but points out that " reversals of water movement in the tracheal conduits are not excluded." We note that there is a fundamental difference between the outlook of those who attribute the underlying mechanism of conduction to diffusion and that of those who attribute it to mass flow. According to the mass-flow hypothesis all the solutes should move in the same direction in the vacuoles of the sieve tubes, unless there are independent movements

in vacuoles and cytoplasm. The diffusion hypothesis assigns independent power and direction of movement to each mobile solute under its own concentration gradient. Mason and Phillis (*194*), by establishing a carbohydrate source above and a nitrogen source below, obtained evidence that nitrogen and sugars might travel in the phloem simultaneously and in opposite directions. This they state is consistent with the diffusion hypothesis, but renders untenable any theory involving a directional mass flow of solute, unless flow is accompanied by a diffusive movement of solutes in the cytoplasm. In further criticism of the mass-flow hypothesis they (195) point out that when sieve pores are partially or wholly blocked by cytoplasm great resistance will be offered to mass flow, and that it is difficult to see how such flow can occur in the Dioscoreaceae, in which compact balls of parenchyma separate sieve tubes of neighbouring internodes.

Finally, there is the question, is conduction a purely physical process or is it a vital process ? There is some evidence that protoplasm plays a part in conduction. For example, Deleano (1911) discovered that the rate at which carbohydrates left green leaves was considerably reduced by treating the petioles with chloroform. More recent experiments indicate that killing by heat has the same effect. It is noteworthy that, although the rate was reduced, migration actually occurred across the killed parts of the petioles. By covering surfaces with rubber bands, wax, oil, etc., Mason and Phillis (*195*) attempted in certain ringing experiments to reduce the oxygen supply to selected lengths of bark. They saw in their results some indications that variation in oxygen concentration might affect the rate of translocation of solutes in the bast, and suggested that the energy released by the respiration of sieve tubes may activate the diffusion of solute molecules in the stationary cytoplasm of these tissue elements. Doubtless this highly important suggestion will stimulate further research.

D. The Lateral Movement of Solutes

First Hartig and then Czapek demonstrated that in incompletely ringed stems conduction proceeds at about the normal rate down narrow bridges, provided these are vertical. With step (p. 141) or oblique bridges conduction was extremely slow. Apparently, therefore, the rapid transfer of food can only occur in a longitudinal direction. Nevertheless, it is clear that some lateral transfer must occur. The process is, however, a slow one. Mason and Maskell, after calculating the rate of the leakage of sugars from phloem to xylem in the cotton plant, concluded that this horizontal movement was akin to ordinary physical diffusion. Doubtless in woody perennials the medullary rays, in addition to acting as tissues for temporary storage, facilitate lateral transfer over short distances. The point to notice is that the internal organization of a plant provides as well for lateral transfer as for the rapid upward and downward movements of water and dissolved solutes.

CHAPTER X

THE GASEOUS EXCHANGES BETWEEN PLANTS AND THE OUTSIDE AIR

A. The Nature of the Gaseous Exchanges [1]

HIGHER plants continually change the composition of the surrounding air by the processes of respiration and photosynthesis. In respiration (chap. XIV), which goes on without interruption in every living cell of a green plant throughout its life cycle, oxygen is absorbed and carbon dioxide is liberated; organic matter is destroyed and the energy liberated is used for vital purposes. In photosynthesis (chap. XIII), cells containing chloroplasts (these will hereafter be called green cells), and only such cells, in the light, but only in the light, absorb carbon dioxide, synthesize carbohydrates, and release oxygen as a by-product. This is the process by which air fouled by the respiration of animals and plants is once more made fit for aerobic organisms to live in.

More than ninety per cent. of the dry matter of a green plant results from photosynthesis. Over a growing period the total amount of carbon absorbed as a result of this nutritive process is greatly in excess of the amount of carbon lost through respiration, in spite of the facts (*a*) that non-green living cells outnumber green cells, and (*b*) that photosynthesis occurs only between dawn and dusk. It is not surprising, therefore, that photosynthesis may mask respiration nearly completely in illuminated green leaves of growing plants, *i.e.*, most of the respiratory carbon dioxide is at once used up again in photosynthesis instead of diffusing out of the leaf.

As a rule, in illuminated tissue containing both non-green and green cells, only one type of gaseous exchange can be easily detected. Whether this will be the photosynthetic type or the respiratory type will depend upon several factors.

(*a*) *The proportion of green to non-green cells in the tissue.* It is difficult to detect respiration in illuminated green leaves because green cells outnumber non-green cells, or to detect photosynthesis in illuminated green fruits, such as green apples and gooseberries, because non-green cells outnumber green cells. (*b*) *The relative activities of the chloroplasts and the respiratory systems.* The chloroplasts of green storage cotyledons, which are packed with food, possess but feeble powers of photosynthesis, while the respiration of the cotyledons is often notable. (*c*) *The light intensity.* Twice a day there occurs for most green tissues a light intensity, termed the compensation point, at which photosynthesis and respiration just balance. (*d*) *The temperature.* In the leaves of winter evergreens on frosty days

[1] Descriptions of the experimental methods used in studying this subject are given in chaps. XIII and XIV.

photosynthesis may be stopped by the low temperature, whereas respiration, although greatly impaired, continues.

B. The Paths of Gaseous Exchange

The gases passing into and out of the submerged parts of aquatic plants, or of the young active respiring parts of the root systems of land plants, are probably in solution, the passage being governed by the laws for the diffusion of solutes (chap. IV, section B). There are no visible discontinuities in the dermal coverings of submerged shoots and roots, but intercellular spaces permit gaseous diffusion to and from the higher levels where stomata or lenticels are present.

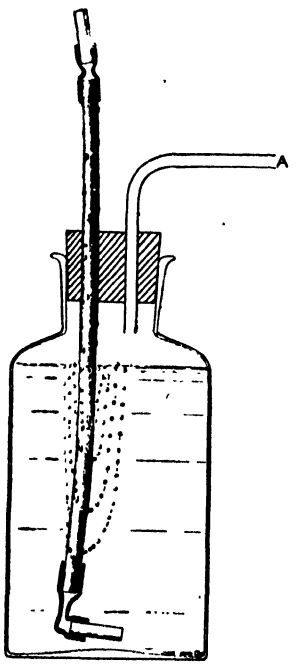


FIG. 20 (see text). As long as suction is applied at A bubbles of air escape from the intercellular spaces through the lenticels. Since bubbling may proceed indefinitely, it is concluded that air (*a*) can enter the cut stem through the lenticels situated above the stopper, and (*b*) can travel downwards in the intercellular spaces to the submerged portion of the stem.

Carbon dioxide and oxygen enter and leave the aerial parts of land plants as gases, although they are used and produced in living cells as solute molecules in solution. Experiments have proved that dermal coverings of cork or cuticle are impervious to oxygen and are not appreciably penetrated by carbon dioxide in low concentrations such as exist in air, and that the ventilating system of green aerial shoots consists of (*a*) stomatal pores, (*b*) spaces between the complementary cells of lenticels, and (*c*) intercellular spaces in the anterior.

Certain facts concerning dermal tissues may be summarized at this stage. The chief function of cork and cuticle is to prevent excessive water loss (p. 111); the plant gains no advantage from their impermeability to gases. Stomata and lenticels primarily serve the plant by making gaseous exchange possible (p. 111); their presence in dermal tissues, however, renders transpiration—a process which may lead to wilting—inevitable (*loc. cit.*). Stomatal movements have a twofold significance: (i.) the narrowing of stomatal apertures is of functional importance when the rate of transpiration is thereby reduced (p. 120); (ii.) the opening movement, at least in the early stages, facilitates gaseous exchanges (p. 158).

The impermeability of cork tissue to gases can be demonstrated by keeping air at different pressures on opposite sides of thin shavings of cork, and it is easy to show that intercellular and outside air communicate by the lenticels, and that the intercellular air spaces are continuous (see fig. 20).

The maintenance of the water level in the vertical tube of a porometer when the glass chamber is affixed to the astomatal surface of a hypostomatal leaf (fig. 160) demonstrates that air cannot pass through cuticle. A more or less rapid fall in level occurs, however, when the glass chamber is affixed to the lower surface. Accordingly, one may infer that air can pass under suction through stomatal surfaces. The rate of fall of the water level, and, hence, that of the passage of air, is governed by the size of stomatal apertures (p. 159). Thus as stomata close in darkened or wilting leaves the rate of fall diminishes, and may become extremely slow. Evidently the rapid entrance of air noted above cannot be attributed to the presence of a permeable cuticle on the lower surface. Hence one may infer that passage is effected through the stomatal apertures. Since a volume of air greater than the total volume of the intercellular spaces of the leaf may pass out under suction, it follows that air can enter the leaf by the stomata outside the glass chamber, and then move in the intercellular spaces of the leaf.

Important experiments, which were performed towards the close of the last century, established the fact that, under natural conditions, gaseous exchanges occur nearly exclusively through stomatal apertures.[1] Stahl covered the lower surfaces of hypostomatal leaves with wax and found that the leaves did not form starch when illuminated in ordinary air. This treatment did not affect the chloroplast mechanism, since starch was subsequently produced in the neighbourhood of pinpricks made in the upper surface. Stahl concluded that the cuticle of the upper surface was impermeable to carbon dioxide, and that this gas under normal conditions diffuses into a leaf through the stomata. F. F. Blackman compared the rates of CO_2 absorption through the upper and lower surfaces of illuminated leaves with the stomatal ratios (*i.e.*, the number of stomata on the upper surface : the number of stomata on the lower surface) for these leaves. Air of known composition was simultaneously passed through small glass-sided gas chambers, which were hermetically sealed by wax to the upper and lower surfaces of leaves, and the issuing gas was analysed. Striking results were obtained for hypostomatal leaves covered with a very thin cuticle (*e.g.*, leaves of virginian creeper, and of plane), carbon dioxide being absorbed at a considerable rate through the stomatal surface, while mere traces passed through the cuticle of the upper surface. According to Blackman, and to Brown and Escombe, other factors were operative in amphistomatal leaves. Thus the average degree of opening of the stomata sometimes differed on the two surfaces, and the greater density of the chloroplasts towards the upper surface caused steeper diffusion gradients at this surface. Allowing for these factors, however, they concluded that the relative rates of absorption closely followed stomatal ratios. Blackman also demonstrated that only negligible amounts of respiratory carbon dioxide diffused through the upper surfaces of darkened hypostomatal leaves, even when the cuticle was very thin, and that the relative rates of diffusion through the upper and lower surfaces of amphistomatal leaves were in direct proportion to the stomatal ratios.

We are not concerned with the behaviour of cuticle towards gases containing high concentrations of carbon dioxide, but we note that experi-

[1] Earlier work and the experiments of Blackman, and of Brown and Escombe, are described by Stiles (*287*).

ments with such concentrations have shown that this gas can penetrate cuticle. Clearly, however, Stahl's and Blackman's experiments indicate that the rate of penetration is negligible when the concentration is 0·03 per cent., as it is in air.

From the experimental results described above one may conclude that during photosynthesis by a green leaf, carbon dioxide diffuses from the outside air, where its concentration is 0·03 per cent., through stomatal pores and intercellular spaces towards the palisade and spongy cells of the mesophyll tissue. This gas then dissolves in the wet cell walls, and diffuses in solution in the direction of the surfaces of chloroplasts, where it combines with water to form carbohydrates. As long as carbon dioxide is thus consumed, a gradient of diminishing concentration will be maintained and diffusion will be continuous. For the oxygen produced the diffusion gradient will be in the opposite direction. Consequently, dissolved oxygen will diffuse away from the chloroplast surfaces, and, upon reaching the wet cell walls, will be liberated as a gas. The concentration of oxygen in the intercellular spaces will thus become higher than that in the air outside, and this gas will therefore pass out through the stomatal apertures. Similarly, during respiration, gaseous exchange between living cells and the outside air occurs through the stomata and lenticels along decreasing concentration gradients of oxygen towards respiring surfaces in protoplasm, and of carbon dioxide away from these surfaces.

C. Intercellular Spaces, and the Composition of the Internal Atmosphere

Microscopical examination shows the presence of intercellular spaces in all parenchymatous tissue. It appears that in land plants these spaces usually occupy more than twenty per cent. and in water plants more than seventy per cent. of the total volume of a leaf. The volume can be measured by determining the change of weight resulting from the injection of a leaf with dilute alcohol (which penetrates more readily than water) of known specific gravity. A detached leaf should be submerged in a little dilute alcohol in a flask from which air is withdrawn by means of a suction pump until there is no further bubbling from the cut surface of the leaf stalk. When air is readmitted into the flask the alcohol will pass through the stomata and inject the intercellular spaces of the leaf.

Gaseous diffusion tends to equalize the concentration of the gases inside and outside the leaf. The processes of photosynthesis and respiration are, however, sufficiently vigorous to maintain marked differences. Thus it has been shown that during photosynthesis the internal atmosphere of green shoots is richer than air in oxygen, and considerable differences in composition have been found for succulent fruits, corms, bulbs, and other bulky organs, in which large numbers of respiring cells are enclosed in dermal tissues that offer considerable resistance to the diffusion of gases. For instance in the carrot, the CO_2 concentration in the interior may rise to over ten per cent., while the oxygen concentration correspondingly falls to approximately the same figure. Certain experiments on stored apples

indicated that these changes in concentration depend upon the intensity of respiration. In the experiments, the rate of respiration was increased by raising the temperature, and the CO_2 concentration rose while the oxygen concentration fell. For example, in one experiment, when apples were stored at 5° C. the percentage of carbon dioxide in the internal atmosphere was 1·5, and of oxygen 19, while with storage at 20° C. the percentage of carbon dioxide rose to 7·5, and that of oxygen fell to 11·5.

The considerations of the last paragraph bear on several important problems. It has been shown that carbon dioxide can act as a narcotic in certain plant processes. For example, Kidd and West (*167*) found that the germination of white mustard seeds can be inhibited by the presence of from two to four per cent. of carbon dioxide in the outside air. It has been suggested that under natural conditions dormancy may be occasioned by the presence of inhibiting concentrations of respiratory carbon dioxide in the intercellular air surrounding the embryo. When seed coats offer resistance to the passage of gases it is not improbable that carbon dioxide will accumulate and act as a narcotic. Possibly under certain natural conditions vital processes may also be arrested as a result of a shortage of oxygen in the intercellular spaces, but there is no definite evidence on this point.

This narcotic effect of carbon dioxide has been exploited by Kidd and West for prolonging the storage lives of fruits. These investigators have shown that respiration is retarded and ripening is delayed when fruits are stored in gas mixtures containing ten per cent. carbon dioxide, ten per cent. oxygen, and eighty per cent. nitrogen. This method of storage is termed gas storage (see Annual Reports of the Food Investigation Board from 1919 onwards).

D. The Rate of Diffusion of Gases through Stomata

After definite experimental proof had been obtained that gaseous exchanges in young shoots occur almost exclusively through stomatal apertures, there arose the problem of accounting in terms of diffusion for the maximum observed rate of CO_2 absorption in photosynthesis under natural conditions. Experiments had shown that a leaf of *Catalpa bignonioides*, when illuminated, absorbed 0·07 ccs of carbon dioxide per sq. cm. of leaf surface per hour from ordinary air, 10,000 parts of which contain only 3 parts of carbon dioxide. It was calculated that the stomata occupied only one per cent. of the total area of the leaf; hence, assuming stomatal diffusion to be the only means of ingress, carbon dioxide was diffusing into the leaf at the rate of over 7 ccs per sq. cm. of stomatal aperture per hour. This was considered to be a surprisingly high rate, seeing that a strong solution of caustic soda, when freely exposed to still air, only absorbs carbon dioxide at the rate of 0·12 ccs per sq. cm. per hour, *i.e.*, nearly sixty times more slowly than an equal area of stomatal aperture. These considerations prompted Brown and Escombe to investigate by means of physical experiments the problem of the rates of diffusion of carbon dioxide through small apertures. Some experiments were performed with septa perforated by single apertures of different sizes, and others with multiperforate septa. In table IV are recorded

results which show the relations of the rates of diffusion to the diameters and areas of apertures.

TABLE IV

Diffusion of carbon dioxide through apertures of various sizes (data from Brown and Escombe)

Diameter of aperture in mm	CO_2 diffusing per hour	CO_2 diffusing per sq. cm. per hour.	Relative areas of apertures.	Relative diameters of apertures.	Relative wts of CO_2 diffusing in unit time.
22·7	0·24	0·06	1·00	1·00	1·00
12·06	0·10	0·09	0·28	0·53	0·42
6·03	0·06	0·22	0·07	0·26	0·26
3·23	0·04	0·48	0·02	0·14	0·16
2·00	0·02	0·76	0·007	0·09	0·10

It will be seen that with increasing diameters (column 1) the rate of diffusion of carbon dioxide increased (column 2), while the rate of diffusion per unit area decreased (column 3). This last result was new to physical science, for it had previously been thought that the rate of diffusion would be proportional to the area of the aperture. Brown and Escombe had discovered the fundamental fact that a single pore allows the passage of less gas in a given time than would a large number of smaller pores having, in the sum, the same area as the large pore. From other experiments with multiperforate septa the conclusion was drawn that if pores were spaced at distances greater than ten times their mean diameter each pore would act independently. Now a cuticularized epidermis with stomata may be considered as a multiperforate septum separating the outside air from the internal atmosphere, and in certain leaves (*e.g.*, those of sunflower) stomata are not much less than ten diameters apart. The marked efficiency of green leaves in absorbing carbon dioxide, as judged by the type of comparison made earlier in this section, can now be readily explained. This comparison was between equal areas (actually 1 sq. cm.) of free surface of caustic soda and of stomatal apertures. But the 1 sq. cm. of stomatal area comprised about 30,000 minute apertures, each of which, according to Brown and Escombe, would act independently, and the results for physical systems indicate that under these circumstances the rate of CO_2 absorption would be much more rapid than with a single pore of 1 sq. cm. This view of Brown and Escombe has in recent years been questioned. Probably the proximity of stomata on leaves leads to reduced rates of diffusion, *i.e.*, the rate of diffusion through any two stomata is less than would be the rate were the stomata twice as distant from one another. There are still many undecided questions of detail concerning diffusion through stomata, but it is improbable

that the general principles that we have briefly considered here will be affected by future work.

If we now compare column 5 with column 6 in table III we see that in these experiments the rate of diffusion was proportional to the diameters of the apertures. This means that the rate of diffusion is doubled by doubling the diameter, while the rate of diffusion per unit area decreases (column 3), since the area is more than doubled.

Brown and Escombe summarized their experimental findings by means of the equation given below. This equation permits the calculation of the volume (Q ccs) of a gas possessing a diffusion constant k, which, in still air, passes per hour through a septum perforated by y pores of mean radius a cm., of mean length L cm., and of mean area A sq. cm., under a pressure difference of $p_1 - p_2$.

$$Q = \frac{k(p_1 - p_2).y.A.3{,}600}{L + \frac{\pi a}{2}}$$

In the physical experiments of Brown and Escombe, L was negligible in comparison with a. Clearly the equation then indicates that the rate of diffusion is proportional to the mean diameter of the pores. L must be taken into account, however, in analysing the results of experiments on leaves, since the length of a stomatal tube may be greater than its width. For photosynthesis by a sunflower leaf in ordinary air, Brown and Escombe collected the following data in order to calculate the volume of carbon dioxide that could diffuse in one hour through the stomata in an area of 1 sq. cm. of a leaf. It was assumed that all the stomata were fully open, and that the air was not moving.

k = (diffusion constant of CO_2) = 0·145 c.g.s. units.
p_1 = (pressure of CO_2 in outer air) = 0·0003 atmospheres.
p_2 was assumed to be nil.[1]
y = (number of stomata per sq. cm.) = 33,000.
A = (mean area of a stoma) = $10^{-7} \times 9{\cdot}08$ sq. cms
a = (mean radius of stomatal aperture) = 0·000535 cms
L = (mean length of stomatal tube) = 0·0014 cms

From these measurements Brown and Escombe calculated that, when fully open, the stomata in 1 sq. cm. of a sunflower leaf could allow the passage by diffusion of 2·1 ccs of carbon dioxide per hour. But the results of experiments on the rate of photosynthesis indicated that, under natural conditions, the rate of CO_2 absorption rarely exceeds 0·2 ccs per sq. cm. of leaf surface per hour. Consequently Brown and Escombe concluded that diffusion through stomata could account for all the carbon dioxide absorbed under natural conditions.

[1] *I.e.*, it was assumed that carbon dioxide immediately upon entering the leaf was used in photosynthesis. Evidently this simplification is open to criticism.

The general nature of the terms employed in the above equation permits us to use it for computing the magnitude of diffusion of any gas, *e.g.*, that of water vapour in stomatal transpiration (p. 120). Brown and Escombe calculated that in a still atmosphere with a relative humidity of twenty-five per cent., fully open stomata on 1 sq. metre of a leaf of a sunflower would allow the passage of 1·7 gm. of water vapour per hour. The maximum rate of transpiration observed was less than 0·3 gm. per sq. metre per hour.

It is probable that these investigators considerably over-estimated the capacity of stomata to allow carbon dioxide and water vapour to pass, and their original equation has been modified by later workers (see Stiles, *287*). The physical and mathematical arguments are too involved for us to consider them here. Accordingly, we have used Brown and Escombe's equation as a convenient summary of the diameter-law, and of the parts played by factors which affect gaseous diffusion through stomata. This procedure would not be justified were it not for the fact that the general conclusions arrived at from Brown and Escombe's equation are still widely accepted.

If for the sake of simplicity we accept Brown and Escombe's results, we may infer that when the stomata of a sunflower leaf are fully open the rate of photosynthesis under natural conditions is never restricted by the size of the stomatal apertures. Now it follows from Blackman's experimental results that the rate of photosynthesis will approach zero during the final stages of stomatal closure.

One is forced to conclude that for every leaf under defined external conditions there is a critical size of stomatal aperture below which the rate of photosynthesis or of transpiration (see p. 120) is restricted by the capacity of the stomata to allow gaseous diffusion. Thoday virtually came to this conclusion when he attributed the diminishing rate of photosynthesis, which he observed in wilting sunflower leaves, to the reduction in the mean size of stomatal apertures. For a given leaf this size will depend upon the concentration of carbon dioxide in the air, the light intensity, the temperature, and other factors which influence the rate of photosynthesis (see chap. XIII). Thus Maskell (*189*), during the course of his work on the photosynthesis of the cherry laurel leaf, found that the rate of photosynthesis may be governed by the size of stomatal apertures when the concentration of carbon dioxide is relatively low and the light intensity relatively high. Alterations in stomatal apertures had, however, no perceptible effect when the light intensity, instead of the CO_2 concentration, limited the rate of photosynthesis. In reviewing earlier work on this subject, Heath (*124*) has pointed out that " with the single exception of the work of Maskell no experimental investigation has yet been published in which the changes in assimilation rate and stomatal

opening were simultaneously followed in the same leaf." He has described an apparatus to be used for this purpose.

We may note in conclusion that the arguments developed in the last paragraph are in accord with the view expressed earlier in this chapter, viz., that the opening phase of stomatal movement should be regarded as of functional value in facilitating gaseous exchange. Clearly, this is only true for photosynthesis between complete closure and the critical size of aperture referred to above.

E. Movements of Stomata

Methods of measuring the dimensions of given stomatal apertures under varying external conditions. (i.) Whole leaves have been observed under the microscope and direct measurements made with a standardized micrometer eye piece. Finding the average size of stomatal aperture on a given leaf surface is a tedious operation, seeing that individual stomatal apertures vary considerably in width. (ii.) Strips of epidermis have been fixed in absolute alcohol and then mounted, before measuring as in (i.). (iii.) For certain purposes, indirect measurements have served. A rapid but rough method is to find out which organic liquids will penetrate into leaves. Stomata must be wide open before ethyl alcohol will penetrate; benzole penetrates when stomata are less wide open; and xylol when stomata are only slightly open. Thus if we find that the penetrative power of one of these liquids increases when a given leaf is transferred from one set of conditions to another, we may infer that the stomata tend to open under the new conditions. (iv.) The rate at which the level of water falls in the vertical tube of a porometer (fig. 21) has often been used as a measure of stomatal size. It appears that the rate is proportional to the square root of the average diameter of all the stomata belonging to the surface under investigation. Besides Darwin's, several other types of porometer have been described (*e.g.*, Knight's and the resistance porometer of Gregory and Pearse). Heath (*125*) has referred to the attempts which have been made to calibrate these various types, and has himself made a critical study of the resistance porometer.

Conditions affecting stomatal movement. It was long ago demonstrated that every normal stoma has the power of opening and closing, but Loftfield (*185*) has in recent years shown conclusively that the behaviour of the stomata of different plants is not uniform. In all plants the following external factors are operative: the water content of leaves—which is governed by the saturation deficit of the atmosphere and also by the supply of available water in the soil—the light intensity, and the temperature. But the relative effect of each factor may vary from plant to plant. There is, moreover, the additional complication that in some plants at least there may occur a rhythmical autonomic movement.

Broadly, we may state (*a*) that if, as generally happens, stomatal apertures alter in width with changing light intensity, they tend to widen with increasing and to narrow with decreasing light intensity; (*b*) that while in most plants stomata actually close for some part of the night, in others closure is a rare event; to this latter class belong

many fleshy-leaved plants and some thin-leaved plants, *e.g.*, the potato; (*c*) that the behaviour of all stomata may, however, be modified by changes in the water content of leaves; thus, in certain leaves, stomata may be found widely open at noon on sunny humid days, and closed at noon on sunny dry days.

The mechanism of stomatal movement. (*a*) *Movements and changes in shape and turgor of guard cells.* Direct observations and measurements under the microscope show that stomatal movements are brought about by changes in the volume and shape of the guard cells. It has long been recognized (see Haberlandt, 109) (*a*) that the expansion and contraction of guard cells must be attributed to the passage of water in and out of these cells; (*b*) that the shape of guard cells changes owing to the unusual thickening of their walls, and (*c*) that peculiarities in the structure of neighbouring subsidiary cells sometimes influence the mode of stomatal movements. Thus for a given stomatal apparatus movements are determined by the turgor pressures of the guard cells and of the subsidiary cells. Imbibitional changes in cell walls or vacuolar colloids might account for the observed facts, but the most generally accepted view has been that, for a given stomatal apparatus, movements are determined by the turgor pressures of the guard cells and of the subsidiary cells. This view has received support from the observation of Heath (*123*) that guard cells collapse when they are punctured. We are thus faced with the central questions concerning the mechanism of stomatal movement, viz., how are the water relations of guard cells and neighbouring cells affected by light intensity on the one hand and by the water content of leaves on the other?

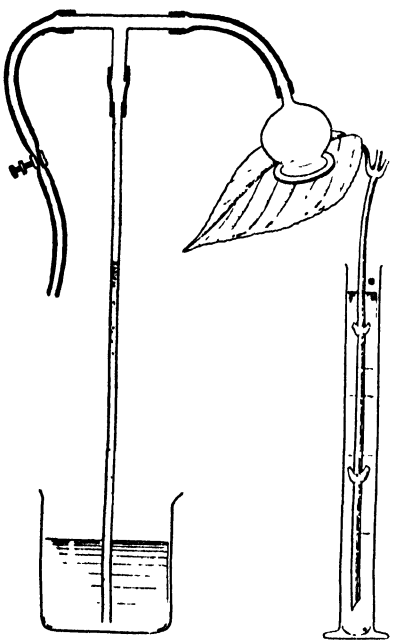


FIG. 21. Darwin's porometer for following changes in stomatal apertures. At the beginning of an experiment the air in the glass chamber affixed to the leaf is under reduced pressure. The rate of fall of the level of water in the vertical tube between two fixed marks provides a measure of the rate with which air passes out under suction through the stomata of the leaf.

Our knowledge of the internal conditions that occasion stomatal movements in wilting leaves is still obscure. There is some evidence that the biochemical phenomena that precede stomatal closure when leaves are darkened (see below) may also participate in causing closure in wilting leaves. Phases of stomatal opening, however, may result directly from the differential shrinkage of leaves as the water content diminishes.

It has now been established that the movements of water that cause the differential changes of turgor pressure in guard cells and subsidiary cells during the slow and gradual processes of opening and closing under varying light intensities, may be attributed to alterations in the osmotic pressure of the sap in the guard cells, and possibly in some species to that of the sap in the subsidiary cells. Wiggans [1] found for a cyclamen leaf that the osmotic pressure of the sap of the guard cells was more than doubled when it attained a maximum between 7 a.m. and 11 a.m., while the osmotic pressure of the sap of the subsidiary cells remained fairly constant and was always less than that of the sap of the guard cells. Iljin had for certain steppe plants previously obtained even more striking figures, viz., 90–100 atmospheres for the guard cells of widely open stomata, and 10–20 atmospheres for those of closed stomata, while the osmotic pressure of the sap of the subsidiary cells remained constant. Strugger and Weber found that in *Galium mollugo* the osmotic pressure of the sap of the subsidiary cells becomes greater during closure than that of the guard cells.

Accordingly one may suppose that during a twenty-four-hour period the osmotic relations of guard cells and subsidiary cells are continually changing. Water will move in the direction of the guard cells as the osmotic pressure (and consequently the suction pressure) increases during the day. When water enters from the subsidiary cells, the volume and turgor pressure of the sap of the guard cells will increase, and because of the differential thickening of the cell walls the shape of the guard cells will change and the apertures will open. The sharp fall in the osmotic pressure at night will be followed by passage of water away from the guard cells. The turgor pressure will thus diminish and apertures will close.

Stomatal movements are not always slow. For example, Gregory and Pearse (*103*) observed an opening movement of the stomata of Pelargonium after one minute's illumination.

(*b*) *Stomatal apertures, fluctuating osmotic pressures, and carbohydrate equilibria.* In attempting to account for the alterations in the osmotic pressure brought about by the action of light, physiologists have attached great significance to the presence of plastids

[1] See Maximov (*196*) and Macgregor Skene (*269*) for further information concerning the work of Wiggans, Iljin, and Strugger and Weber.

(usually chloroplasts) and starch in the guard cells of stomata that are capable of movement. Moreover, chloroplasts are absent from the other epidermal cells of the higher plants, and starch is not usually produced by these cells. Opinions differ concerning the composition of the plastids in the guard cells of the white parts of variegated leaves. Some observers have maintained that chlorophyll is present in small amounts, and others that the plastids are free from chlorophyll.

The rise in the osmotic pressure of the sap of illuminated guard cells was formerly attributed to the production by photosynthesis of osmotically active substances. Doubtless this happens under natural conditions when guard cells contain chloroplasts. But stomata in the white parts of variegated leaves may also open by day and close by night, and it has been found that stomata in general can open and close in the absence of external carbon dioxide. Evidently a simple photosynthesis hypothesis does not explain all the facts. It must be remembered that big increases of osmotic pressure must be accounted for. Feeble photosynthesis by pale green chloroplasts could not bring these about. Green guard cells could, of course, compete with the mesophyll cells in using such respiratory carbon dioxide as had been stored in the intercellular spaces. Consequently, some photosynthesis would take place, but this would soon cease in leaves placed in air free from carbon dioxide.

The clue which has led to the modern interpretation of events was discovered by Lloyd. He found that carbohydrate equilibria in both green and non-green guard cells differ from those in ordinary green cells, in that the amount of starch in a guard cell increases by night and decreases by day. These important observations have been confirmed many times (see, *e.g.*, the results of Loftfield's experiments, which are shown in graphical form in fig. 22). It has been inferred that light either directly or indirectly promotes the hydrolysis of starch to sugar in these cells. Consequently, the osmotic pressure of the sap of illuminated guard cells will rise, independently of the presence of chlorophyll, provided that starch is present in these cells. The reconversion of sugar to starch in the dark will be accompanied by a lowering of osmotic pressure.

(*c*) *Light, hydrion concentration, and carbohydrate equilibria.* We are left with the problem of accounting for the carbohydrate transformations that have been observed in guard cells under varying light intensities. Sayre, in 1926, showed that the apertures of the stomata of *Rumex patientia* can attain about half their maximum size when leaves of this plant are placed in the dark in air containing ammonia vapour, and that the stomata can be made to close, even in the light, in an acid atmosphere. He suggested that the position of carbohydrate equilibria in guard cells is governed by the *pH* of the

sap in these cells, and attributed the actual variations in *pH* that he observed to the effects of respiration and photosynthesis.[1] He suggested that in the dark the accumulation of respiratory carbon dioxide causes a decrease in the *pH* of weakly buffered sap, and that in the light, owing to photosynthesis by green guard cells, the respiratory carbon dioxide is used up, and, consequently, the *pH* increases. Clearly he had proposed a new form of photosynthesis hypothesis to account for stomatal movement. But this hypothesis did not explain why an external supply of carbon dioxide and the presence of chloroplasts in the guard cells are not essential factors for the opening movement. Scarth (*260*) has put forward a more comprehensive theory on the basis of his own and of the earlier

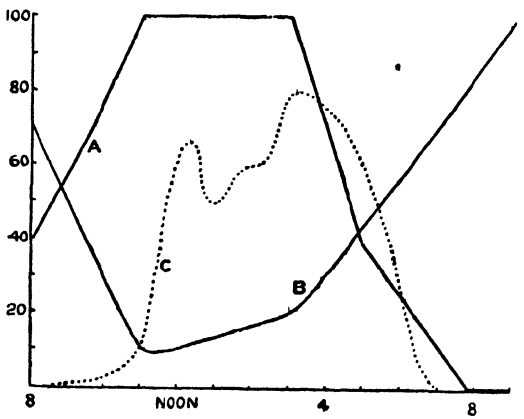


FIG. 22. Graphs showing changes during a 12-hour period in (A) the relative widths of the stomatal apertures in the lower surface of a leaf of the Lombardy poplar, (B) the relative amounts of starch in the guard cells of this leaf, and (C) the intensity of sunlight. (From Loftfield (*185*), modified.)

experiments of Sayre and others. In this theory he maintains that any response of the guard cells to the *direct* action of light is small, and attributes stomatal opening in the light to the increase in the *pH* of the sap of the guard cells that results from the general reduction of the CO_2 concentration in the intercellular spaces of the leaf. Evidently such an alteration in the composition of the internal environment of guard cells must always accompany photosynthesis by mesophyll tissue. Scarth considered that in variegated leaves the effects of photosynthesis by the green parts would spread some

[1] We may note that Sayre also reported that during stomatal closure in wilting leaves the *pH* in the guard cells changes, and starch is synthesized. Scarth (*260*) obtained similar results. Evidently the sequence of changes that lead to stomatal closure in wilting leaves may possibly be the same as that which occurs in darkened turgid leaves (but see p. 161).

distance into the white parts, *i.e.*, it was not essential that the guard cells themselves should be green. Nevertheless, he found that stomata situated some distance away from the assimilating tissue were only slightly affected by changes in light intensity. He confirmed the fact that the absence of carbon dioxide does not prevent, and, indeed, may favour stomatal opening in the light; and he concluded that the essential condition for opening is not that continuous photosynthesis should occur, but that respiratory carbon dioxide must not accumulate, *i.e.*, the sap of the guard cells must not develop acidity. According to this view, opening would occur independently of the presence of carbon dioxide in the outside air, provided the light intensity were sufficient for photosynthesis to balance respiration. As soon as respiration becomes the more vigorous process, carbon dioxide accumulates, acidity rises, and stomatal closure is induced.

Scarth has made numerous experimental investigations, particularly on the behaviour of the stomata of *Zebrina pendula* (*Tradescantia zebrina*). He has shown that only those wavelengths of light which are most strongly absorbed by chlorophyll are appreciably active in causing stomatal opening. He obtained evidence that stomatal movements are accompanied by changes of *pH* in the guard cells as well as by reversible carbohydrate transformations. For example, he found by Small's range indicator method (see p. 464) that in passing from darkness to light the *pH* may rise from about *pH* 5 to between *pH* 6 and *pH* 7, or perhaps higher. The fact that changes of a similar order and in the same sense occurred in the intercellular spaces lent support to his view that it is the general reduction by photosynthesis of the CO_2 concentration in the leaf that occasions stomatal opening, and its general increase by respiration that occasions closure.

Scarth also found that when he placed epidermal sections of a leaf in dilute ammonia (*pH* 7·3) the amount of starch in the guard cells diminished even in the dark, and stomatal apertures widened; while in dilute acetic acid there was no reduction in the amount of starch, and stomatal opening was inhibited.

Results that are more informative have recently been reported by Small and Maxwell (*272*). For example, the natural *pH* of leaves of *Coffea arabica* varied between 4·4 and 5·2 in the dark, and between 5·9 and 6·2 in the light. The maximum possible change was thus found to be nearly *pH* 2·0, which was greater than that which occurred in most of the other plants that were examined. In Coffea leaves placed in buffer solutions maximum opening was found at *pH* 5·7 (acetate buffer), and 6·6 (phosphate buffer). The influence of the buffer on the maximum should be noted. In some of the tests starch was absent from the guard cells at *pH* 5·7 to 5·9 (acetate buffer),

and at *pH* 6·7 to 7·2 (phosphate buffer). Small and Maxwell point out that amylases from different sources have different *pH* optima, but note that salivary amylase has activity optima at *pH* 5·6 in acetate buffer, and at *pH* 6·6 in phosphate buffer. By taking into account buffer effects, they have thus obtained clear evidence for suggesting that change in the *pH* of the guard cells, such as might be brought about by alternating light and darkness, results in change in amylase activity, and consequently in carbohydrate equilibria.

Summary. The facts that have been recorded in this section concerning stomatal movements in the light and in the dark may be summarized thus :—

Darkened Guard Cells.[1]	Illuminated Guard Cells.
Respiratory carbon dioxide accumulates in the intercellular spaces.	Respiratory carbon dioxide contained in the intercellular spaces used up by the mesophyll.
pH of the guard cells shows a tendency to fall.	*pH* of the guard cells shows a tendency to rise.
The increasing acidity favours the formation of starch from soluble sugars.	The decreasing acidity favours the hydrolysis of starch.
The osmotic pressure of the sap of the guard cells decreases.	The osmotic pressure of the sap of the guard cells increases.
Water leaves the guard cells, and their turgor pressure and volume decrease.	Water enters the guard cells, and their turgor pressure and volume increase.
The guard cells change their shape, and stomatal apertures narrow.	The guard cells change their shape, and stomatal apertures widen.

In conclusion it must be pointed out that several workers have inferred from their experimental results that, in addition to carbohydrate transformations, swelling of colloids or changes in permeability may be concerned in stomatal movements. " With increased permeability, turgor diminishes and the guard cells approach each other ; conversely, when impermeability is again restored, turgor increases and the stoma opens " (Maximov, *196*, p. 177).

[1] Or wilting leaves, after the first stomatal opening brought about by differential shrinkage (see footnote, p. 163).

PART III

NUTRITION AND METABOLISM

CHAPTER XI

GENERAL SURVEY OF PROBLEMS OF METABOLISM

A. The Nature and Sources of the Food of Green Plants

The essential elements of macronutrients. Until the significance of the gaseous exchange shown by illuminated green leaves (p. 151) was understood, it was generally accepted that most of the food material necessary for the growth of rooted plants is derived from the brown humus of the soil. Even when it was realized that the absorption of carbon dioxide from the air by green leaves is a nutritive process, enriching the plant in carbon compounds, the possibility was not excluded that the soil might supply additional and even essential organic foods. It cannot be too strongly emphasized that before the humus theory of plant nutrition could be set aside, plants had to be made to pass through their life cycles without receiving any organic matter from the environment. By the middle of last century success was achieved (see Sachs, *256*, Russell, *255*). As a result of water culture and sand culture experiments it appeared to be well established that the carbon dioxide of the air can act as the sole source of carbon for green plants (a conclusion which is supported by recent experiments, see p. 352), and that the root system need only be supplied with solutions of minerals containing the following elements: nitrogen, sulphur, phosphorus, potassium, magnesium, calcium, iron. In the absence of any one of these elements the growth of shoots or roots or both was stunted, pathological effects (*e.g.*, chlorosis, premature withering of leaves) became apparent, and the plants died before flowering. Some authorities added chlorine to the list of these elements, which, with carbon, hydrogen, and oxygen, were grouped together as the essential elements.

In table V the composition of two culture solutions, which were much used in the last century, is given, and a few remarks are made about culture solutions in general. It will be noticed that all the elements excepting iron and, possibly, chlorine are supplied in concentrations sufficiently high for them to be described as essential elements for macronutrients. As we shall see shortly, traces of other elements besides those named in table V are essential for at least some plants.

TABLE V. *Composition of solutions for the water-culture of green plants*

Sachs' solution.		Knop's solution.	
Potassium nitrate	1·00	Calcium nitrate	0·80
Calcium phosphate	0·50	Potassium nitrate	0·20
Magnesium sulphate	0·50	Potassium dihydrogen phosphate	0·20
Calcium sulphate	0·50	Magnesium sulphate	0·20
Sodium chloride	0·25	Ferric phosphate	trace
Ferrous sulphate	trace		

Grams of inorganic salts dissolved in 1,000 c.c. distilled water.

N.B. The culture solution should be continually aerated. It should also be periodically renewed to guard against such alteration of physiological balance among the constituents as might result from the unequal absorption of ions. Among the other factors that must be taken into account are the osmotic pressure, and the hydrogen ion concentration (see Miller, *201*).

The metallic and acidic ions shown by culture experiments to be essential must be present in soils in which rooted plants grow. These ions are derived from the chemical weathering of mineral matter, the bacterial decay of humus, and from the fixation of atmospheric nitrogen (chap. V). It should be noted that ammonium salts as well as nitrates may be present in soil solution, and that some plants grow equally well in culture solutions containing ammonium salts as in solutions containing nitrates. It is the concern of agricultural science to investigate methods of manuring soils with elements (particularly nitrogen, phosphorus, and potassium) in which they happen to be deficient and to determine the effects of manuring on plant yield. We note here that the knowledge gained by experiments on the growth of plants in sand culture or water culture has played and still is playing an important part in developing the practice of mineral manuring. Fundamental physiological researches, the results of which may later have important practical applications, are being carried out in order to ascertain at what stage or stages of development or reproduction each essential element plays its part; in what way the element affects events occurring in the tissues of a growing plant (for a short discussion, see p. 174); and how, in exercising their functions, the essential elements may interact one with another (Gregory, *102*, Richards, *246*).

The occurrence in plants of non-essential elements. It has long been known that elements such as sodium, aluminium, and silicon, which are important constituents of soils, may occur in plants in relatively high concentrations, although they are not essential for growth and reproduction. On occasions, however, they may serve a growing plant beneficially (*e.g.*, see p. 180).

From time to time the presence in plants has been reported of most of the elements, common or rare, known to occur in the mineral

constituents of soils. Clearly, provided the molecules or ions containing such elements are present as solutes in soil solution, and provided membranes are permeable to those molecules or ions, there is no reason why they should not be absorbed and translocated with the essential elements. Moreover conditions in certain plants and environments may favour the accumulation in these plants of an element which occurs in very low concentration in the soil solution. For example, Trelease has reported that certain plants may absorb and accumulate selenium in sufficient concentration to poison browsing animals. The question arises, are any of the elements that occur in low concentrations in soil solutions to be considered as beneficial or, perhaps, essential for the growth of plants ?

The trace elements (essential or non-essential) of micronutrients.[1] It is probable that impure inorganic salts were used in all the earlier water culture experiments, and that little regard was paid to such traces of substances contained in glass vessels, etc., as might dissolve in the water of the culture solution or as might originally have been present in the seeds from which the culture plants were reared. Great importance now attaches to the possible presence in nutrient solutions of such additional elements, for it appears that " an element may not be dismissed as unessential if it is present in concentrations greater than one in a billion." Mazé reported in 1914 that the healthy growth and development of certain plants required the presence in the culture solutions of low concentrations of zinc, boron, aluminium, and silicon. Using highly purified inorganic salts, and employing silica vessels for the preparation of distilled water and for holding the culture solutions, many investigators have found that macronutrients containing the essential elements that have been listed above were insufficient to support a plant throughout its life cycle. Healthy growth and development were not observed unless traces of salts containing other elements were added to the culture solutions. In excess these salts caused injury or death. Particular attention has been paid to boron, manganese, zinc, and copper, as possible essential trace elements to be supplied in what have been termed micronutrients (for bibliography, see Miller, *201* ; Brenchley, *48*, Shive and Robbins, *266*). For instance, Brenchley and Warington have reported that " broad beans, grown in nutritive solutions composed of salts *spectroscopically free from boron*, fail to complete their development, . . . whereas with the addition of traces of boric acid normal growth is made." Concentrations from 0·1 to 40 parts per million were effective ; concentrations of 200 parts per million caused injury. Attempts to substitute other elements for boron were unsuccessful. Similar evidence has been obtained suggesting

[1] For further information see Stiles, W., "Trace Elements in Plants and Animals," published at the University Press, Cambridge, 1946.

that boron is essential for the growth of a number of other plants (*e.g.*, tomato, sugar beet). Zinc is now included among the micronutrients since it has been found that certain plants show poor growth, and that others suffer from "deficiency diseases" (*e.g.*, rosette disease of apple shoots), in the absence of this element; but, according to Chandler, it is usually present in sufficient amounts as an impurity in macronutrient salts. McHargue has reported that some of his experimental plants, especially those belonging to the Leguminosae, did not develop in healthy fashion in the absence of manganese. Inadequately supplied with manganese, Algerian oat suffers from a deficiency disease called grey-speck; lack of manganese in a soil in which this oat is grown has been corrected by treating the soil with solutions of manganous sulphate. It is not yet generally accepted that copper is an essential trace element, but the fact that it is found in organic combination as copper proteid in certain oxidation enzymes supports the view that it may be essential for some plants. Stout and Arnon (*297*), who give a clear account of the steps they took to maintain pure culture solutions of known composition, inferred from their experimental results that copper as well as zinc and manganese may be essential for the growth of tomato plants. The distilled water alone, taken from a tin-lined copper still, was found at times to supply sufficient copper for the promotion of growth. Excepting in very dilute solutions, copper salts are extremely poisonous.

Other elements, present in soil solution in minute quantity, without being essential, may nevertheless favourably influence the growth of plants. Arnon (*5*) found that poor growth was made by asparagus and lettuce when these plants were reared in culture solutions in the absence of any trace elements. Moreover, symptoms of a deficiency disease were apparent. These were not observed, and growth was more vigorous when the culture solution contained minute amounts of boric acid, manganous chloride, zinc sulphate, and copper sulphate. The inference may be drawn that all the essential elements of macronutrients and micronutrients were then present. When, in addition, the plants were fed with a solution containing traces of salts of molybdenum, vanadium, chromium, nickel, cobalt, tungsten, and titanium, further stimulations of growth were observed, and these were remarkably great in the experiments with lettuce. Arnon suggests that one or more of these elements may play a part in the nutrition of the higher plants. Evidently, there lies ahead a vast field for further investigation of the actions, beneficial and harmful, exerted by trace elements in soil solutions on the growth, development, and reproduction of rooted plants. It must be remembered that our knowledge is still insufficient for us to state that any one of these trace elements is essential for all plants.

B. The Chemical Composition of Plants

We shall here regard the whole green plant or any of its parts as a mixture of chemical compounds, which may, like any other mixture, be subjected to qualitative and quantitative chemical analysis. As a rule the first step taken in an analysis is the determination of the percentage of water present from the difference between the fresh weight and the dry weight. This has been found to fluctuate from fifty to ninety per cent. in leaves and from thirty-eight to sixty-five per cent. in freshly felled timber, while in air-dry seeds it may fall as low as ten per cent. The residual dry matter, with which we are specially concerned here, may then be analysed to determine its elementary and molecular composition. An immense amount of data has already been gathered, particularly for crop plants, and there is abundant scope for further work, for it appears that the elementary and molecular composition of whole plants and of equivalent organs and tissues varies in different species growing in the same environment, in the same species growing in different environments, and in the same species at different stages of development.

The elementary composition of plants. Evidently the essential elements of macronutrients and micronutrients must necessarily be present in all plants; and, as has been pointed out on p. 167, compounds containing many other elements may also be absorbed from the environment.

The relative quantities of the different elements in plants vary considerably. The bulk of the dry matter of plants is composed of organic compounds. The amount of carbon fluctuates around forty-five per cent., that of oxygen around forty-five per cent., and of hydrogen around five per cent. The percentage of nitrogen is extremely variable. It depends upon the organ analysed, and changes during development. The amount of nitrogen in the dry matter of some tissues is less than one per cent., but in that of others it may be as high as ten per cent. The remaining elements may be collectively considered as constituents of the plant ash inasmuch as they are not lost by heating the dry matter until all the carbon has burned away. Plant ash usually represents about five per cent. of the total dry matter of plants, but in certain tissues (*e.g.*, wood) the amount may be less than one per cent.

The molecular composition of plants. Since we discuss in some detail in Appendix I the chemistry of the principal organic compounds found in plants, it will suffice at this stage to record the names and elementary composition of some of these compounds. The extreme chemical heterogeneity of plants, and the complex nature of many of the components, should be noted.

CLASSIFICATION OF CHEMICAL COMPOUNDS WHICH HAVE BEEN FOUND IN GREEN PLANTS

INORGANIC COMPOUNDS. (i.) *Water.* (ii.) *Inorganic salts:* nitrates, phosphates, sulphates, chlorides, etc., of potassium, calcium, magnesium, iron, etc.

ORGANIC COMPOUNDS. (i.) *Compounds containing C and H :* carotin. (ii.) *Compounds containing C, H, and O :* carbohydrates (pentose and hexose sugars, disaccharides, pentosan and hexosan polysaccharides) ; fats and fatty acids ; waxes ; sterols ; xanthophyll ; terpenes ; flavones, anthocyanins, tannins, and other aromatic glycosides ; lignin ; pectic substances which may also contain calcium ; the vegetable acids (sometimes free but often as metallic salts) ; ascorbic acid ; and a wide range of other aliphatic and aromatic alcohols, acids, esters, ketones, and aldehydes, and of homocyclic and heterocyclic phenols. (iii.) *Compounds containing C, H, O, and N :* proteins (usually also contain sulphur and sometimes contain phosphorus), and their derivatives (peptones, polypeptides, and amino-acids) ; lecithins (which also contain phosphorus) ; porphyrins, of which chlorophylls *a* and *b* also contain magnesium, and hæmochromogens (*e.g.*, cytochrome) also contain iron ; purines ; nucleic acid and nucleotides (which also contain phosphorus) ; some glycosides (*e.g.*, indican, and amygdalin and other cyanogenetic glucosides) ; alkaloids ; and a variety of simple amines and amides.

Some of the classes of compounds (*e.g.*, those found in protoplasm, cell walls, and the recognized food substances) named in the above list occur universally in plants. Other substances (*e.g.*, glycosides, waxes, terpenes, alkaloids, purines) are not widely distributed. Sometimes the differences are between different species in the same genus, *e.g.*, *Eucalyptus australiana* and *Eucalyptus phellandra* contain very different relative proportions of the two terpenes, cineole and phellandrene (Baker and Smith, *10*). Furthermore, for certain substances (*e.g.*, anthocyanins) varietal differences occur within a single species.

It should be noted that the qualitative composition of a given species may be affected by environmental stimuli. For example, chlorophyll is, as a rule, only produced in the presence of light, and when the environment can provide iron salts. Again, it is known that the presence or absence of anthocyanins in certain flowers (see p. 343) is determined by the temperature. And it is common knowledge that the development of scent and flavour of the fruit of popular varieties of apple (*e.g.*, Cox's orange pippin), pear, grape, etc., is strongly influenced by soil and climatic factors.

Usually more than ninety per cent. of the dry matter of plants consists of organic compounds. It is clear from the results of analysis (for example, see table VI) performed on the assumption that these compounds are exclusively present as carbohydrates, fats, protein, and wood and fibre, that much variation in the quantitative composition of certain comparable fully grown organs occurs from plant to plant.

During growth, the percentage composition continually changes. For example, the concentration of the components of wood and fibre increases during the growth of trees.

TABLE VI. *Percentage composition of the dry matter of certain vegetable foods.*

	Carbohydrate	Fat.	Protein.	Wood and fibre	Ash
Wheat grain . . .	79	2	14	2·8	2·2
Broad bean seed . .	58·5	1·8	28	8·2	3·5
Coconut (excluding the shell)	17·2	71	10	0·8	1

C. The Distribution of Chemical Compounds within Plants

Knowledge of the location of various substances may be gained by direct observation (*e.g.*, for pigments), macrochemical analysis of tissues or of collected sap, and microchemical methods (*i.e.*, chemical methods used in conjunction with a microscope). Substances may be conveniently classified as occurring in protoplasm, vacuoles or the cell cavities of dead cells, or cell walls.

Substances in protoplasm. In addition to those compounds which appear to form part of the living machinery (p. 8), plastic and other substances which, though often essential for protoplasmic activity, are not part of the living machinery, are frequently located in protoplasm. The most easily recognizable of these substances are the solid starch grains found in chloroplasts or leucoplasts. Protein grains and crystals, and liquid drops of fatty oils, have also been observed in protoplasm. Further, the water in which the protoplasmic micellæ are dispersed always contains in solution various nutrient inorganic salts and organic substances. It appears that inorganic solutes are not always uniformly distributed in living cells (p. 11).

Vacuolar substances. Mineral salts and sugars (*d*-glucose, *d*-fructose, and sucrose) are invariably present in vacuolar sap, and organic acids (such as malic, tartaric, oxalic, succinic, citric, etc.) frequently occur either free or as salts. Among the other substances which have been detected are the pectins, inulin, many glycosides (including flavonic glycosides and anthocyanins), polyphenols, tannins, amides (*e.g.*, asparagine), proteins, amino acids, and alkaloids. Some of these substances (*e.g.*, the tannins) are widely distributed in the higher plants, and some (*e.g.*, inulin) occur in but few species. In many succulent plants, mucilages are distributed as slimy masses in cells.

Some of the products mentioned in the last paragraph, and others also, are occasionally found in specialized cells (see Haberlandt, *109*). Thus, essential oils, resins, gums, and mucilages, occur in secretory glands. These glands are sometimes external and sometimes internal. External glands secrete substances to the exterior of plants ; internal glands either secrete substances to the cavities between cells or produce their secretions and then degenerate. Other specialized cells that are occasionally found are the sacs which act as the repositories for resins, tannins, and crystals.

Soluble carbohydrates (*e.g.*, cane sugar), nitrogenous substances, and inorganic salts (*e.g.*, potassium salts), have been found in the cell sap of sieve tubes, and organic food substances as well as inorganic salts may be present in the sap which travels in xylem vessels and tracheides.

Cell wall substances.[1] Cellulose forms the basis of all cell walls, and its presence may be detected by means of chlor-zinc iodine, which stains cellulose blue, and by its solubility in zinc chloride plus hydrochloric acid and in other solvents. Pectic substances occur in association with cellulose in all parenchymatous tissue. They are not affected by the reagents used in the detection of cellulose. They can be recognized by the facts that they are soluble in ammonium oxalate, and are stained by ruthenium red. It seems to be generally accepted that the middle lamella of cells, which is the first part of the cell wall to be laid down after mitosis, is composed of calcium pectate. Much of the pectic substance in cell walls is ordinary pectin (soluble pectin or pectinogen). But some pectic substance is present

[1] See Onslow (*214*, chap. II).

as insoluble pectin (pectose or protopectin), which may enter into combination with metallic salts of calcium, magnesium, or iron, or become associated with cellulose as pectocellulose.

Pectic substances tend to form gels with water, as may be shown by adding an aqueous extract of young clover leaves or carrots, or any other tissue containing pectase, to a pectin sol. The gel formed is more easily seen if a calcium salt is present in the solution. It is possibly owing to the physical properties of this gel that parenchymatous cells cohere to form tissues. There is some evidence that the flesh tissue of certain fruits becomes mealy as they grow old because this gel is converted by the enzyme protopectinase into soluble pectin. This substance is often abundantly present in the cell sap of ripe fruits (*e.g.*, red currant).

During the differentiation of permanent tissues from the cells which are formed by the activity of meristematic tissues, the composition of the cell alters, either as a result of chemical change brought about by secreted enzymes, or as a result of the secretion of substances that become incorporated within the existing cellulose micellæ in the walls, or are deposited in layers on the original cellulose framework.

When dermatogen differentiates into epidermis, a medley of substances classed under the name cutin is produced. On the exterior, cutin forms a layer, continuous save for the stomatal pores, which is called cuticle. Underneath the cuticle, cutin and cellulose are intimately associated in varying proportion as cuto-cellulose. The cutin component of epidermal walls is insoluble in the solvents used for cellulose, but a part may dissolve in alkalies. It is stained brown with chlor-zinc iodine, and takes up Sudan III and other lipoid stains. The mixture of substances called suberin is produced during the differentiation of cork tissue from phellogen. The general properties of suberin are similar to those of cutin. Priestley and his co-workers have shown that the Casparian band (fig. 9) in young roots and in certain stems (*e.g.*, Potamogeton) contains substances akin to cutin and suberin.

Tissue elements with lignified walls are found in primary and secondary xylem, phloem (*e.g.*, the secondary phloem of the lime stem), the cortex (*e.g.*, the sclerenchymatous masses and rings which occur in many monocotyledonous stems), and the pericycle (*e.g.*, in the stems of sunflower and the vegetable marrow). In different elements lignification of walls occurs to different degrees. Thus it is relatively slight in the annular and spiral vessels of protoxylem, and profound in sclerenchyma. Lignin is the essential component of lignified tissue and reacts in lignified cell walls to give a yellow colour with aniline chloride, and a red colour with phloroglucinol and hydrochloric acid. Lignin occurs in association with cellulose as lignocellulose in lignified tissue, the amount present depending on the extent of the lignification of lignified walls. Usually over fifty per cent. of the dry weight consists in cellulose and between twenty-five and thirty-five per cent. in lignin : furthermore, fats, resins, gums, hemicelluloses, tannins, and colouring matter, may be present.

Besides occurring in woody elements hemicelluloses have been found in pericarps and testas, and in the walls of the storage tissues of seeds, shoots, and roots. Hemicelluloses do not dissolve in acid zinc chloride. They are soluble in dilute alkalies, which, it should be noted, have no effect on cellulose proper.

Other cell wall components of occasional occurrence are waxes, gums, mucilages, and resins. Insoluble inorganic substances are represented by silica and calcium oxalate. Grains of silica are sometimes found in epidermal cell walls, *e.g.*, those of certain grasses and cereals, and of

horsetails. This may be shown by burning a transverse section on a glass slide in the presence of strong sulphuric acid ; the cell walls will be represented in the residue as skeletons of silica. Cluster crystals of calcium oxalate are occasionally to be seen hanging from cell walls. Similar clusters are sometimes contained in specialized crystal sacs.

The subject-matter of Appendix I permits the classification of cell-wall substances by chemical criteria, as follows : (*a*) *Substances with polysaccharide affinities :* cellulose, pectic substances, hemicelluloses, mucilages, gums. (*b*) *Substances with fatty oil affinities :* cutin and suberin. (*c*) *Higher paraffins, alcohols, esters :* waxes. (*d*) *Aromatic compounds :* lignin, resins. (*e*) *Mineral substances :* silica.

D. The Functional Importance of the Substances found in Plants

Protoplasm and skeletal substances. We note at the outset that the constituents of the protoplasm in a living cell (p. 8) form part of a metabolizing system which can produce plastic substances,[1] and manufacture substances of physiological and ecological importance. In the course of metabolism waste products may also arise. It is probable, however, that most of the substances that occur in a plant perform some useful service at some time in the life cycle. Protoplasm, raw materials for metabolism, and metabolic products, are all accommodated within the skeletal framework of cell walls. These structures are composed of simple and compound celluloses. Special functions are served by certain cell wall substances. For example, the pectin in cell walls causes cells to cohere (see p. 173). Again, the great mechanical strength of sclerenchymatous tissue may be ascribed to the presence of lignin in the cell walls. It is important to notice that water, by creating turgor in cells, contributes to the mechanical resistance which shoot systems and root systems offer to stresses and strains. Mineral salts (whether essential or non-essential) and organic compounds in crystalloid solution contribute to the development of the osmotic pressures on which the absorption of water depends. Evidently suberin, cutin, and other dermal coverings (*e.g.*, resins, waxes, and gums), by restricting water loss, help to maintain turgor, and belong to the class of skeletal substances.

Substances of physiological importance. We shall include in this class such substances as play a part in the events concerned with the internal economy of plants.

Water. A brief statement will be found on p. 71.

Inorganic salts. All soluble salts make important contributions to the total osmotic pressure possessed by cell sap. Some insoluble minerals (*e.g.*, silica) that contain a non-essential element may have a protective function (p. 180). Here we shall consider the special functions of the inorganic salts containing essential elements

[1] This term denotes metabolic products which take a further share in metabolism during growth processes subsequent to their formation (see Sachs, *257*).

(p. 166). These salts, together with carbon dioxide and water, serve as the raw materials from which are manufactured the organic compounds known as foods. During growth these foods migrate to meristematic and developing regions of shoot and root, and are there converted into the organic substances that are found in the protoplasm, cell walls or vacuoles of these regions. Inorganic salts may also be used for anabolism in these regions.

It is instructive to consider the distribution of essential elements among the anabolic products (p. 171, and appendix I) out of which the various parts of cells in these regions are constructed. Thus nitrogen is present in most of the substances found in protoplasm. It occurs with sulphur in proteins, thiamine, and biotin; with phosphorus in lipoids, nucleic acid, and cophosphorylase, the codehydrases, and certain other coenzymes; with sulphur and phosphorus in cocarboxylase; with iron in the porphyrin groups of hæmochromogens (*e.g.*, cytochrome, catalase); with copper in copper proteids (*e.g.*, in the polyphenol oxidase of potato, and, possibly, ascorbic oxidase); and, in the green pigments of chloroplasts, with magnesium. Considering these facts alone we can understand why an adequate external supply of salts containing nitrogen, phosphorus, sulphur, iron, magnesium, and possibly copper, is essential for the continuous manufacture of protoplasm, and, consequently, for the growth, the development of full vegetative activity and the reproduction of a green plant. It is recalled that certain of these elements (*e.g.*, copper, see p. 169) must be supplied in very low concentrations. Apparently, for all the elements, this supply need not necessarily be a constant one, since plants may accumulate salts very readily during the earlier stages of a growth process, and use the surplus later, provided, of course, the salt containing an essential element can migrate in the plant. It is an important problem to determine for crop plants, vegetables, and fruit trees, the best time to supply to a soil an element in which it happens to be deficient.

It is clear that knowledge of the chemical composition of the functioning systems in green plants, of the activities possessed by these systems, and of the relation of these activities to other activities, may help in the interpretation of the visible effects (stunted growth, physiological disease, premature withering, etc.) seen in experiments on plants reared in water cultures or sand cultures (pp. 166, 168) deficient in an essential element. As a further example let us consider the effect of mineral nutrition on the development of respiratory activity. Inasmuch as certain oxidation systems (pp. 29 to 44) are constructed from nitrogenous organic compounds that may also contain sulphur, phosphorus, iron, or possibly copper, it is not surprising that a deficiency in one of these elements, but apparently not in potassium (Gregory, *102*), may result in a reduction

in respiratory intensity and in the diminished vigour of all the physiological processes governed by the liberation of respiratory energy, *i.e.*, of all processes of growth and development. Possibly this is also the reason why iron is necessary for the *production* of chlorophyll, a fact demonstrated long ago by water culture experiments; but it should be noted that the absence of iron from the chlorophyll molecule was a more recent discovery. Chlorosis seen in plants reared in solutions deficient in phosphates or sulphates may be similarly attributed to impaired oxidative activity. In accounting for chlorosis when nitrogen is deficient we must remember the additional fact that this element is essential for greening because it is a component of the chlorophyll molecule, *i.e.*, nitrogen resembles iron, phosphorus, and sulphur, in that it contributes to the development of oxidative activity, and magnesium in that it provides one of the component elements of the chlorophyll molecule.

Direct inferences concerning the functions of certain essential elements may similarly be drawn from such knowledge as we possess of the chemical composition of substances occurring in cell walls or vacuoles. For example, one of the functions of calcium is to combine with pectic acid in the formation of the calcium pectate contained in the middle lamella of a cell wall.

In addition to providing elements found in metabolic products of functional importance, essential macronutrients and micronutrients yield ions which may, in the free state, govern processes that take place in meristematic, differentiating or permanent tissues. Thus it has been suggested that boron has a catalytic function in mitosis; but certain workers believe that boron is continuously consumed during growth, and therefore cannot strictly be regarded as a catalyst. It is known that magnesium and phosphate ions act catalytically in zymase cleavage: these ions are present in the free state when cleavage is complete. It should be noted that such catalysts may act, not only during the growth and development of the functional units of plants, but also later when some of these fully developed units are exercising their functions.

Potassium deserves separate consideration. It is a mobile element, moving in the xylem and the phloem, and is found in all parts of a plant, with considerable accumulations in the leaves and meristematic cells. It is absent from nuclei and plastids (p. 11), but may accumulate on the surfaces of these structures. Potassium salts of vegetable acids may occur in vacuoles, but organic compounds containing this essential element have not been detected among the components of protoplasm. James and Penston have suggested, however, that it may combine with proteins. But there is little doubt that it is active in the ionic form; and many functions have been attributed to potassium ions. Some authorities, but not all (see Gregory, *102*),

consider that potassium promotes protein synthesis in meristematic regions and elsewhere. According to Briggs (*50*) the photosynthetic activity of chloroplasts is depressed if leaves develop in soils deficient in potassium; he considers that this element promotes the development of activity in the protoplasmic factor (p. 246). On the other hand, the rate of respiration per unit mass of tissue shown by barley leaves does not appear to be much affected by deficiency of potassium, except when this is extreme (Gregory, *loc. cit.*). A number of other special catalytic actions of potassium in metabolism have been reported. It may promote the production by leaves of polysaccharides, *e.g.*, starch and fructosans; and it may bring about an increase in the amount of diastase formed by a plant.

Culture experiments have shown that vegetative development is favoured by abundant potassium nutrition. Manuring with potassium salts soils deficient in this element leads to the production of a greater number of leaves, and, also, to an increase in the average area of a leaf. Warne (*321*) reported that, in his experiments on seakale beet, increase in area probably resulted from an increase in the size of cells and not from the production of a greater number of cells. He inferred that the potassium supplied must have been operative during the period of cell expansion. An important point to notice is that potassium feeding slightly enhanced the water content per unit area of leaf. Warne also observed that potassium did not affect the total number of stomata produced during the development of leaves, *i.e.*, the frequency of stomata was greater in the leaves of plants reared in soils deficient in potassium, since the average area of such leaves was less. In experiments with pot plants he found that transpiration was proportional to stomatal frequency, and concluded that potassium manuring may, as a consequence, reduce the rate of transpiration, and "affect all phases of the physiology of the leaf in which gaseous exchange through the stomata plays a part."

Warne's experiments on transpiration are of particular interest because Wallace and others have observed that the leaves of apple trees and of other plants wither prematurely, and show effects known as scorching, when they receive an inadequate supply of potassium, suggesting that under certain conditions potassium may affect the water content of leaves. This point, however, has not been firmly established (see Warne, *loc. cit.*; Gregory, *loc. cit.*). Evidently, water content depends upon the balance between the rate of conduction of water to a leaf and the rate of transpiration. Warne (*322*) made experiments on apple shoots from trees receiving abundant potassium, and from others receiving a poor supply of this element. He found that potassium deficiency not only brought about an increase in stomatal frequency (and possibly, therefore, in transpiration per unit area), but also a decrease in the ability of shoots to conduct water; he suggested that these causes may contribute to the susceptibility of apple trees to drought conditions when receiving an inadequate supply of potassium.

Owing to the practical importance of mineral nutrition it is not surprising that a great body of miscellaneous information is available concerning effects, visible from the outside of plants or from a microscopical examination of sections, that result from a shortage in

the supply of potassium, or, indeed, of any of the essential elements. Miller (*201*) treats extensively the subject of the functions of elements absorbed from the soil, and gives a considerable bibliography. More recent work is described in reviews such as the one written by Richards (*246*). Bearing on this subject is an admirable summary by Wallace (*320*) entitled "The Diagnosis of Mineral Deficiencies in Crop Plants."

Organic food substances. Plastic carbohydrates (*e.g.*, starch, inulin, hemicelluloses, cane sugar and hexoses), proteins and their derivatives, and fatty oils, are usually regarded as constituting the group of substances known as organic foods. They may be immediately available for use in the cells in which they are detected; for example, the sugars which are always present in meristematic regions participate in respiratory oxidations and in the synthesis of the constituents of protoplasm and cell walls (pp. 180 and 341); but cane sugar, amino acids, and other migratory substances, found in the phloem or elsewhere, may be diffusing to regions in which they will later be used.

Reserve foods demand more detailed consideration here.

Before a substance is classed as a reserve food it must be shown that a preliminary period of accumulation is followed by a period in which the substance is maintained *in situ* at a relatively high concentration; and that later, in association with physiological processes taking place in the immediate vicinity or elsewhere, the concentration of the substance diminishes. These conditions are satisfied for all the substances we shall mention below. Storage of food may be (*a*) transitory, as in the formation of starch in many green leaves in the light, and in certain tissues where differentiation is about to take place; or (*b*) of longer duration, as in hibernating organs. Grains of starch or protein, globules of oil, and hemicellulose thickenings of cell walls, may occur in the endosperm or in the cotyledons of seeds, and in the storage parenchyma of shoot systems and root systems of herbaceous, shrubby and woody perennials. In swollen underground storage organs, reserve carbohydrates are frequently dissolved in cell sap. Inulin occurs in colloid solution in certain species belonging to the Compositæ, *e.g.*, in the tubers of the Jerusalem artichoke. Sucrose, glucose, and fructose, exist in crystalloid solutions in onion bulbs, and in the turnip, beet, carrot, etc. The mobilization by enzymic hydrolysis of insoluble foods and of such foods as exist in colloid solutions in cells, is discussed elsewhere (p. 207).

Quantitative researches in Trinidad have pointed the way to testing by modern chemical methods the fallible eye judgments of greater or less made after viewing microscopical preparations, which had for so long been relied on in the study of storage in stems and

roots. Mason and Maskell (see p. 146) found that, during the vegetative development of the cotton plant, an acid-hydrolyzable polysaccharide accumulated in the lower regions of the stem, especially in the bark, *i.e.*, there existed a negative concentration gradient from the leaves downwards for this polysaccharide. A negative gradient in the bark was also found for total nitrogen, and this was mainly determined by the crystalloidal component, asparagine (fig. 19, p. 147). The inference drawn is that amino groups, which are all-important to the plant for the synthesis of proteins, are stored in molecules of asparagine. Moreover, the evidence suggests that tissue elements in the bark store foods that are produced in excess of immediate requirements for vegetative development. But, as Mason and Phillis (*193*) point out, " the only criterion of storage is redistribution," and their quantitative data showed that as flowering proceeds and bolls develop in the cotton plant, nitrogen migrates to the reproductive regions from the bark of the vegetative parts.

Other plastic organic substances. There occur in plants a large number of plastic organic substances (*e.g.*, glycosides, free aromatic compounds, vegetable acids) that are not usually regarded as foods. Some of them may possess ecological importance ; but their functions are often obscure. Great interest attaches to the suggestions that have been made concerning the functions of malic, tartaric, and other vegetable acids. The part they may play in metabolism is discussed in chap. XV. Here we note that in " acid plants " free ammonia, produced by protein breakdown or by the reduction of nitrates, is rendered harmless by the production of ammonium salts of these acids. In " amide plants " or " non-acid plants," ammonia, which is a toxic substance, is prevented from accumulating by a special form of metabolism that leads to the production of asparagine or glutamine. Illustrative examples are given by McKee (*197*) and Chibnall (*59*).

Hormones. Clearly substances that correlate the activities of separated regions (see p. 364) should be included in the group of substances of physiological importance.

Substances of ecological importance. In this class we shall include the substances whose functional value may be related either to a special physical condition of the environment (*e.g.*, excessive dryness of the soil or air), or to the presence of other organisms in the environment. The production of large amounts of cutin or of additional dermal coverings (*e.g.*, of wax) may serve to reduce the rate of transpiration. The pentosan mucilages produced in the interior of succulent xerophytic plants belong to this class, since they increase the water-holding power of cells. It has been suggested that the essential oils secreted by certain leaves may bring about a diminution in the rate of diffusion of water vapour, and consequently of stomatal transpiration.

Cutin and suberin are always of ecological importance in that they protect plants from invasion by parasites and from the depredations of insects, snails, etc. It has been suggested that silica may occasionally serve as a protective substance, since experiments have shown that snails eat grass devoid of silica in preference to grass containing this substance. A similar protective function against snails has been attributed to the cluster crystals in cell walls. Some of the glycosides, alkaloids, tannins, etc., may be distasteful or poisonous, and so protect certain plants from being eaten by animals, or invaded by parasitic fungi and bacteria.

The pigments and scents of flowers and the secretions of nectaries are of considerable significance when they act as attractive substances in cross pollination by insects, etc. Pigments, scents, and foodstuffs in the feeding tissue of fleshy fruits belong to this class when distribution of seeds is effected by birds. The statements made concerning the relations which exist between animal behaviour and chemical substances in plants are usually based on the observations which have been made in the field by naturalists.[1]

Waste-products of metabolism. We place amongst waste-products of metabolism those substances for which we fail to find a function. A good example is the anthocyanin in the swollen root of the red beet. Some of the substances secreted into ducts, sacs, etc., may be waste products.

E. The Division of Metabolic Labour within Cells and among Tissues

Highly diverse chemically active systems exist side by side in unicellular organisms such as Chlamydomonas or yeast. In multicellular organisms showing differentiation, in addition to division of metabolic labour within single cells, striking differences are shown in the metabolism of the various living tissues. Metabolism ceases in cells which die during differentiation. Thus vessels, tracheides, fibres, and cork, do not directly function in the metabolic activity of the plant.

The anabolism of meristematic tissue. In zygotes, meristematic apices, embryonic cells, and cambia, occur the syntheses from inorganic salts and

[1] Extracts from the section entitled "Utilitarian Doctrine, how far true: Beauty, how acquired," from the "Origin of Species," are to the point. Darwin writes: "Flowers rank amongst the most beautiful productions of nature; but they have been rendered conspicuous in contrast with the green leaves, and in consequence at the same time beautiful, so that they may be easily observed by insects. *I have come to this conclusion from finding it an invariable rule that when a flower is fertilized by the wind, it never has a gaily coloured corolla.* . . . A similar line of argument holds good with fruits; that a ripe strawberry or cherry is as pleasing to the eye as to the palate . . . will be admitted by everyone. But this beauty serves merely as a guide to birds and beasts, in order that the fruit may be devoured and the manured seeds disseminated: *I infer that this is the case from having as yet found no exception to the rule that seeds are always thus disseminated when embedded within a fruit of any kind (that is, within a fleshy or pulpy envelope), if it be coloured of any brilliant tint, or rendered conspicuous by being white or black.*" The italics are ours, and are used to show that Darwin's assignment of functions to colours, scents, and feeding tissues, followed observations in the field.

organic foods translocated to these regions of at least some of the substances found in protoplasm (see p. 8), and of the cellulose and pectic components of cell walls (see below). Conceivably a fraction of the substances found in protoplasm are synthesized elsewhere, and are translocated to the meristematic regions. But at least the final phase in the formation of specific proteins and of other insoluble or colloid components must take place in the meristematic cells. It has now been conclusively proved (see p. 205) that root tips, when supplied with thiamine, can manufacture from sugars and inorganic salts all the substances produced during the growth of roots. It may well be that similar remarkable powers of synthesis are possessed by all meristematic cells.

The anabolism of differentiating tissue. (i.) *Cell walls.* During differentiation the production of pectic substances and cellulose continues, and as a result of other forms of carbohydrate metabolism, hemicelluloses, gums, and mucilages, may become incorporated in the cell walls ; or metabolism may lead to the production of (*a*) aromatic compounds such as lignin (*e.g.*, in differentiating xylem) and the resins (*e.g.*, in the bud scales of Pinus), or (*b*) derivatives of fatty acids such as cutin (*e.g.*, in differentiating epidermis) and suberin (*e.g.*, in differentiating cork). The cell wall is, " to a certain extent, the non-living record of some of the metabolic activities of the protoplasm " (Onslow, *214*, p. 66). Since cell wall substances are not diffusible, at least the final stages in their synthesis must occur in the cells in which the substances are located. Moreover, arabinose, xylose, mannose, and galactose, are represented in many of these cell wall substances. For instance, galactose, galacturonic acid, and arabinose, occur in pectic substances. Now the only sugars which migrate in plants are glucose, fructose, and cane sugar. Accordingly we may infer that intramolecular changes among carbohydrates may take place in the cells in which cell walls are being laid down.

(ii.) *Substances in protoplasm and vacuoles.* Since pigmented substances do not migrate in plants, it follows that at least the final step in the production of plastid and vacuolar pigments takes place in the cells in which the pigments accumulate during differentiation. Moreover, direct observation shows that plastid pigments are actually produced in the plastids, and that vacuolar pigments originate in the vacuoles. We have no room to extend these arguments to include substances, such as alkaloids, terpenes, etc., that can be recognized by applying microchemical tests.

The anabolism of vacuolated living cells of differentiated tissues. The power of photosynthesizing monosaccharides from carbon dioxide and water is confined to specialized green cells, and is displayed pre-eminently by the mesophyll. Most of the other syntheses in plants occur independently of light, and may take place either in green or in non-green cells (see chap. XII, section B). It is probable that all vacuolated cells possess powers of anabolism. When such powers lead to the accumulation of proteins, carbohydrates, and fats, we refer to the cells as storage parenchyma. Since starch, hemicelluloses, and protein grains, occur in the solid state, and since inulin exists in colloid solution in cell sap, we may infer that the final stages in the synthesis of these storage products must actually occur in the storage tissues. On the other hand, for a storage product such as cane sugar, which exists in crystalloid solution, it is difficult to decide whether the reserve food is synthesized by the storage cells, or whether it is synthesized elsewhere and translocated to the storage cells. It is not yet known to what extent differentiated cells (*e.g.*, companion cells) retain the remarkable powers of utilizing mineral salts possessed by meristematic cells. It is a significant fact that certain differentiated cells (*e.g.*, pericycle when produc-

ing roots in cuttings) can be stimulated by hormones to become meristematic Latent powers of anabolizing mineral salts may, therefore, be retained.

The division of catabolic labour among tissues. All living cells respire; but in a single plant different types of substance may serve as respiratory substrates, and different modes of respiratory oxidation may be displayed. For instance, in young germinating seedlings of the sunflower, fats may be oxidized in the cotyledons, while sugars are being oxidized in the plumule and radicle. Evidently there is a division of oxidative labour among tissues. Again, different substances undergo hydrolysis in different parts of a plant. Fats are hydrolyzed in the cotyledons of the sunflower seed, and starch undergoes hydrolysis in the leaves of the independent seedling. It is not yet clear whether the occurrence of different types of oxidation or of hydrolysis should be entirely attributed to the occurrence of different substrates in various parts of the plant, for it is possible that the enzymic constitution of protoplasm may also be variable. Our knowledge of the distribution of oxidative and hydrolytic enzymes in the tissues of higher plants is far from complete. Thus we know very little about the catabolic powers of phloem, medullary rays, and the cortex. The disappearance of a food substance from a storage tissue during the germination of seeds or the sprouting of buds, and from green leaves in the dark, may, however, be ascribed with confidence to the activity of hydrolytic enzymes. It is probable that in most of these hydrolyses, the substrate and the specific enzyme are located in the same cell. Nevertheless, it is possible that certain enzymes migrate from one tissue to another. For example, there is some evidence [1] to show (*a*) that the mustard-oil glycosides contained in the testa of seeds of *Lunaria biennis* are hydrolyzed by enzymes produced by the cells of the cotyledons, and (*b*) that diastase is secreted by the scutellum during the germination of cereal grasses, and cytase by the cotyledon during the germination of date seeds.

F. Types of Biochemical Change, or the Chemical Powers of Protoplasm

Every living cell possesses diverse chemical powers, whereby many different types of constructive and energy-yielding processes are effected. The elucidation of these powers constitutes a fundamental branch of the analytical study of metabolism. A complete description of the metabolic events that accompany and govern growth and development might be given did we know exactly what types of chemical change every living tissue in a growing organism is capable of effecting, and the specificity of each effective system. The systematic study of enzymes (chap. III, section C) has thrown much light on such catabolic powers of living cells as *hydrolysis, phosphorolysis, glycolysis, oxidation, reduction, deamination, deamidation,* and *decarboxylation.* There are, however, but few anabolic events which can as yet be definitely attributed to enzyme activity (*loc. cit.*). Apart from these few, the manifold constructive chemical events in growing organisms must for the present be attributed to the extensive anabolic powers of the whole protoplasm, or of specialized parts such as chloroplasts.

[1] See Haas and Hill (*108*).

The chemical powers displayed by specialized green cells in photosynthesis. The power of photosynthesizing carbohydrates from carbon dioxide and water belongs exclusively to specialized cells containing chloroplasts. During photosynthesis there is created a linkage between carbon atoms that persists in practically all other organic compounds in plants, and, consequently, in most other living organisms. Since oxygen is evolved, the overall reaction may be described as a *photoreduction*. Inasmuch as photosynthesis is a complex process, accomplished in stages which have not yet been studied in isolation, the synthetic powers possessed by chloroplasts cannot yet be precisely described.

As a rule when chemists, using symmetrical substances, synthesize compounds containing asymmetric carbon atoms, they arrive at a racemic mixture containing *d*- and *l*-optical isomers in equal amounts. It is a noteworthy fact, however, that in biosynthesis only one of a pair of optical isomers is usually produced. Protoplasm thus possesses the power of *asymmetric synthesis*, a power acquired by chemists only in recent times. Doubtless the stereochemical configurations of the first formed sugar (probably *d*-glucose) and of the sugars into which it is converted are respectively determined by specific metabolizing structures in the chloroplasts, and in the protoplasm as a whole. As a result metabolism is directed by protoplasm along definite stereochemical lines,[1] and the number of carbohydrate metabolites circulating in living organisms is thereby limited. Thus, out of sixteen possible aldohexoses, only *d*-glucose, *d*-mannose, and *d*-galactose occur in plants. It appears that under the conditions prevalent in living cells these three aldohexoses, and the ketohexose, *d*-fructose, are readily interconvertible (see Ruhland and Wolf, *253*). One may therefore assign to protoplasm the power of effecting *intramolecular changes*. Apparently, optical inversion does not accompany such changes in the hexose group of carbohydrates. The production of *d*-fructose diphosphate by yeast during fermentation, independently of the hexose used, may be cited as an example. It should be noted that in the hexose diphosphate, fructose is in the active or γ-form. This is also its state when combined in cane sugar. Hence one may infer that protoplasm can *activate* fructose (*i.e.*, change fructopyranose into fructofuranose). The reverse change,

[1] Although the metabolism of substances containing asymmetric carbon atoms appears frequently to be directed in nature along certain fixed channels, it does not necessarily follow that protoplasm could not act on each of the two optical isomers, were both to be produced. Thus it is well known that when mould fungi are fed with the ammonium salt of racemic tartaric acid, the *d*-salt is first attacked, but later the *l*-salt is also destroyed. Further, it should be noticed that the optical isomers and the racemic variety of a given substance may all be found as natural products. The occurrence of *d*-, *l*-, and *dl*-mandelonitrile, in cyanophoric glucosides from different species of plants affords a good example (see p. 434).

viz., *deactivation*, occurs spontaneously when the ketohexose is liberated by hydrolysis.

On anabolism in general. As a result of photosynthesis not only green cells but all the living cells in a plant are provided with sugars as well as with the water, gases, and mineral salts, which are absorbed from the environment. It is probable that all forms of protoplasm possess the power of oxidizing sugars and their cleavage products. Recondite biochemical problems abound concerning the types of change which lead to the formation from sugar of other metabolic products containing carbon, hydrogen, and oxygen, and to the introduction of elements such as nitrogen, sulphur, phosphorus, iron and magnesium, into organic compounds. We have only room here to consider a few of these problems.

A complex study may be simplified by sorting out the substances participating in anabolism into classes, such as inorganic anabolites, primary organic anabolites, secondary organic anabolites, and anabolic end products. The term *inorganic anabolite* denotes one of the raw materials (carbon dioxide, water, mineral salts) absorbed from the environment, or an inorganic salt (nitrite, ammonium salt, sulphide) produced by reduction. We shall use the term *primary organic anabolite* to represent such organic compounds, which are not themselves products of condensation, as can undergo condensation and participate in the formation of anabolic end products. It will be pointed out below that this term may be applied to hexose sugars, the sugar acids and alcohols, hexosephosphates, cleavage products of hexoses or of hexosephosphates, and to certain amines, amides, and other substances.

The molecules of *anabolic end products* may be very big (*e.g.*, proteins, polysaccharides and substances, such as pectins, having affinities with polysaccharides, cutin and suberin, lignin, tannin, and resins), or relatively small (*e.g.*, cane sugar, gallic acid, ascorbic acid, nucleotides, biotin, and pantothenic acid), or of intermediate size (*e.g.*, fats, chlorophylls *a* and *b*, lecithin, sterols, carotinoids, nucleic acid). Certain of these end products are readily decomposed (*e.g.*, by hydrolysis), and yield well-defined classes of substances the molecules of which are of greater size than those of the primary anabolites. It has for a long time been widely held that such decomposition products may actually serve as building stones from which the anabolic end products concerned are constructed. Thus amino acids have been spoken of as the building-stones for proteins, and monosaccharides for polysaccharides; fatty acids and glycerol for fats; fatty acids, phosphoric acid and choline for lecithin; higher aliphatic acids for suberin and cutin; gallic acid or protocatechuic acid for tannins; phenolic alcohols for lignin; and terpenes or phenols for resins. Apart from the monosaccharides and glycerol, none of the organic

building-stones mentioned belongs to the class primary organic anabolite. Condensations involving sugars or cleavage products of sugars are necessary for their formation. To emphasize this fact we propose to substitute the term *secondary organic anabolite* for building-stone. Evidently by so doing we clarify the problem of analysing the anabolic sequences that occur in the formation of hydrolyzable end products. In any such sequence we must determine the types of change undergone and metabolic powers displayed in passing from inorganic anabolites to anabolic end products by way of primary organic anabolites, and secondary organic anabolites. It is realized that intermediate metabolities may be formed in passing from a primary to a secondary anabolite, but we think that at present it is sufficient to recognize these two classes, and the antithesis between the qualifying epithets, primary and secondary, will serve this purpose.

Non-hydrolyzable anabolic end products may be formed by the condensation of primary anabolites without the intermediate production of secondary organic anabolites. Thus it is a noteworthy fact that terpenes, carotinoids, sterols, and certain aromatic substances, appear to be constructed from compounds containing two and three carbon atoms (see also p. 415), and that flavones and flavonols, anthocyanidins, and certain other aromatic compounds, are constructed from primary anabolites with three and six carbon atoms. There is evidence which suggests that the synthesis of many *hydrolyzable anabolic end products* (*e.g.*, fats, proteins) progresses by way of secondary anabolites. Cane sugar, however, is presumably produced from glucose and fructose, and starch may be synthesized from the primary anabolite, glucose-1-phosphate (p. 63). The alternative paths of synthesis may be schematically illustrated thus :—

Inorganic anabolites	⟶	Primary organic anabolites.	⟶	Anabolic end products.
		Secondary organic anabolites.	⟶	

In the succeeding sections of this chapter we shall consider the chemical nature of the substances which are included in the four classes named above, and the types of change by which passage from substances in one class to those in a higher class is effected. It is a striking fact that although different anabolic end products are synthesized in immense numbers by plants, the products may be sorted out into relatively few chemical classes (*e.g.*, proteins, polysaccharides, fats, tannins, etc.). It appears that synthesis is directed by protoplasm along certain well-defined lines which are relatively few in number. There are good reasons for supposing that the primary organic anabolites do not constitute a numerous group. The variability among the anabolic end products formed from these

primary compounds may be attributed to permutations and combinations of a limited number of protoplasmic activities (each of which will show specificity), and, consequently, of types of chemical change. Evidently, during the course of anabolic sequences, greater variation of chemical structure will be found among members of the more complex anabolic products. Thus individual substances belonging to the group anabolic end products (*e.g.*, proteins) will be more numerous than those belonging to the secondary anabolites (*e.g.*, amino acids) from which the end products have been synthesized.

The formation of primary organic anabolites. It will be convenient to assume here that hexose sugars are directly produced in photosynthesis by the condensation of inorganic anabolites (viz., carbon dioxide and water), and that they can exist as open-chain compounds. Since they participate in *condensations* which are accompanied by *dehydration* (p. 430), they may be classed as primary organic anabolites. It has been suggested that they (or the corresponding acids or alcohols produced from them respectively by *oxidation* and *reduction*) may also participate without cleavage in metabolic transformations that yield inositol and phenols, flavones and flavonols, anthocyanidins, and other aromatic compounds, but there is a complete lack of experimental evidence. Hanes (see p. 63) has shown that by *condensation* with *dephosphorylation* starch may be produced from glucose-1-phosphate, which is, therefore, a primary organic anabolite.

Pentose sugars and their derivatives may be produced by the *decarboxylation* of hexuronic acids, and may possibly serve as primary anabolites in the formation of certain glycosides (*e.g.*, nucleosides) and pentosans. It is recalled that ribofuranose and its derivative desoxyribofuranose are always present in protoplasm. The nucleic acids are now named after them (p. 443), and the former is a component of codehydrases, cophosphorylases, and other coenzymes that participate in controlling metabolism. If we have correctly depicted the origin of pentoses, we may conclude that during growth protoplasm must use its powers of oxidizing hexoses and of decarboxylating the product of oxidation. To realize that working parts of ultramicroscopic dimensions, and possessing such special metabolic powers, exist is an essential first step in the direction of describing in chemical terms the events occurring in growth. The biochemist's dogma is that in due course all the chemical powers described in these pages, whether used before, during or after a growth process, will be attributed to enzymic systems with determined specificities.

Hypotheses concerning the formation from sugars of vegetable acids containing four, five, or six carbon atoms, are described in chap. XV and summarized in fig. 1. In the postulated changes *glycolysis*, *decarboxylation*, *oxido-reduction*, and *condensation* (including

CO_2 assimilation), play a part. Bennet-Clark (*21*) has discussed the view that vegetable acids are the building stones from which some of the complex products (*e.g.*, amino acids, alkaloids) originate. Chibnall (see pp. 201-4) has considered more recent evidence concerning the production and consumption of vegetable acids in linking carbohydrate and protein metabolism. In fig. 1 are indicated possible methods of the formation of alanine, and of aspartic and glutamic acids and their acid amides, asparagine and glutamine. *Amination* and *amidation* are the protoplasmic powers displayed.

The enzyme zymase may be present in all living plant cells. In cleaving, probably aerobically as well as anaerobically, hexoses in the presence of phosphates (see fig. 2), it displays powers of *phosphorylation*, *transphosphorylation*, *glycolysis*, *oxidoreduction*, *isomerisation*, *dehydration*, and *decarboxylation*, and may occasion the production of highly reactive compounds. Some of the compounds with three carbon atoms are phosphates (*e.g.*, triosephosphates, phosphoglyceric acid, phosphopyruvic acid), but may give rise to others which are not (*e.g.*, glycerol, methylglyoxal, pyruvic acid, lactic acid). The products containing two carbon atoms are acetaldehyde and ethyl alcohol. It is now a widely favoured theory that one of the functions of zymase cleavage is that of producing primary anabolites. It has long been held that it is instrumental in converting hexoses into labile substances that can undergo respiratory oxidations. Inasmuch as anabolic events are endothermic it is reasonable to suppose that they are coupled with exothermic changes, such as oxidations or the conversion of active phosphate linkages to ester phosphate linkages (p. 53). A simple example of anabolism involving products of zymase cleavage is the production of ethyl esters and metabolic products containing ethoxyl groupings. In these ethyl alcohol presumably acts as a primary anabolite. In fig. 1 it is indicated how pyruvic acid may take part in the production of oxalacetic and other C_4 acids, cisaconitic and other C_6 acids, and alanine. It may also (see p. 189) be involved in the formation of fatty acids.

Methyl esters (*e.g.*, chlorophyll, pectin) are invariably present, and compounds containing methoxyl groups (*e.g.*, certain anthocyanins, and alkaloids) are widely found in green plants. It is possible, therefore, that methyl alcohol is one of the primary anabolites containing a single carbon atom, but there is no evidence concerning its origin. Chemists attribute the presence of the dioxymethylene group in certain alkaloids (*e.g.*, narcotine), and of the methyl-imino-group in others (*e.g.*, cocaine), to condensations in which formaldehyde participates. Thus this compound may have considerable anabolic significance quite apart from the rôle it has, from time to time, without any valid experimental evidence, been alleged to play in the photosynthesis of carbohydrates.

It has been suggested that urea and amino derivatives of products of zymase cleavage containing two and three carbon atoms may be important primary nitrogenous organic anabolites. As a prelude to their production, absorbed nitrate must be reduced to ammonia, probably with an intermediate nitrite stage, and there is some evidence that these reductions are effected by enzymes. In passing, we note that urease has been shown to act synthetically. It is possible, therefore, that urea may be produced by the union of ammonia and carbon dioxide in any living cell containing urease. The amount of carbon dioxide absorbed by plants for this synthesis would be trifling in comparison with that used in the photosynthesis of carbohydrates.

No suggestions have yet been made concerning the nature of primary anabolites containing sulphur. Absorbed sulphates must be reduced before compounds such as allyl sulphide can be produced.

The condensation of primary organic anabolites. To account for the formation from primary organic anabolites of open-chain and cyclic secondary organic anabolites (*e.g.*, fatty acids, amino acids), or anabolic end products (*e.g.*, phytol, carotin, melanin), one must assign powers of *condensation* to protoplasm. Such condensation may be effected either by *addition*, as happens when two unsaturated compounds combine, or by *substitution*. The latter operation will be accompanied by the liberation of water, ammonia, or some other substance.

(i.) *The formation of open-chain compounds. Chain extension* accompanies the formation of most of the aliphatic acids, alcohols, and hydrocarbons found in plants, and one infers that protoplasm can cause the combination of two distinct molecules, but not necessarily of different compounds, by uniting them through carbon atoms. Chemical evidence suggests that the fusion, with or without dehydration, of two aldehydes or of an aldehyde and a ketone, may be responsible for the production of long-chain aliphatic compounds from primary organic anabolites.[1] Thus, for example, butyric acid might be formed from acetaldehyde by means of an aldol condensation, followed by an oxidoreduction :

$$\underset{\text{acetaldehyde.}}{CH_3CHO} + CH_3CHO \rightarrow \underset{\text{acetaldehyde aldol.}}{CH_3 . CH(OH) . CH_2 . CHO} \rightarrow \underset{\text{butyric acid.}}{CH_3 . CH_2 . CH_2 . COOH}$$

or the aldol might condense with acetaldehyde to give straight-chain compounds with six, eight, ten, etc., carbon atoms. Smedley and Lubrynska suggested that chain extension might be brought about

[1] The only biochemical evidence which one can adduce in support of this suggestion is the fact that Neuberg and his co-workers discovered that acyloin, a keto-alcohol (probably $C_6H_5CH(OH) . CO . CH_3$), was synthesized when benzaldehyde was added to a solution of sugar that was undergoing fermentation in the presence of yeast or maceration extract. Neuberg ascribed the synthesis to the condensing action of an enzyme, which he termed carboligase, on the added benzaldehyde, and the acetaldehyde produced by zymase cleavage.

as a result of the condensation with dehydration of acetaldehyde and pyruvic acid (equation (i.)). The unstable higher unsaturated ketonic acid so formed would immediately undergo decarboxylation (equation (ii.)) :—

(i.) $CH_3 . CHO + CH_3 . CO . COOH \rightarrow CH_3 . CH : CH . CO . COOH + H_2O$
(ii.) $CH_3 . CH : CH . CO . COOH \rightarrow CH_3 . CH : CH . CHO + CO_2$
(iii.) $CH_3 . CH : CH . CHO + O \rightarrow CH_3 . CH : CH . COOH$

The resulting unsaturated aldehyde might then be oxidized to a C_4 unsaturated acid (equation (iii.)), or by condensing with pyruvic acid and by a repetition of the other stages yield a C_6 unit. Evidently straight-chain unsaturated aliphatic acids of high molecular weight, each with an even number of carbon atoms, would be produced by a succession of condensations and of the other changes described above. The corresponding saturated aliphatic acids would, of course, be readily formed from unsaturated acids by reduction.

It would be simple by considering different primary anabolites to extend these theoretical notions to account for the formation of aliphatic acids with an uneven number of carbon atoms or with branched chains and of hydroxy acids or ketoacids corresponding to amino acids such as valine, serine, and lysine.

It should be noted that in the transformation of carbohydrates to aliphatic acids the state of the molecule becomes progressively reduced as the carbon chain lengthens. *Reduction* proceeds even further in the production of higher alcohols (*e.g.*, phytol, xanthophyll, carnaubyl alcohol), and hydrocarbons (*e.g.*, carotin).

Two examples of chain extension are included in fig. 1. It is postulated that oxalacetic and other C_4 acids are produced by the condensation of pyruvic acid and CO_2, and that cisaconitic acid and other acids belonging to the citric group arise from the union of pyruvic and oxalacetic acids.

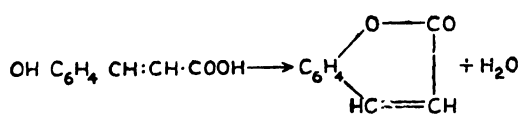
Some evidence now exists (p. 201) that amino acids are produced from α-ketoacids. Condensation with ammonia may lead to *imination* followed by *reduction*. In this way amino acids such as aspartic acid and glutamic acid may be produced (see fig. 1). These may then donate amino groups to other ketoacids (*e.g.*, pyruvic acid) ; so giving rise by *transamination* to other amino acids (*e.g.*, alanine). Powers of *amidation* are also possessed, as is evidenced by the production of asparagine and glutamine from aspartic acid and glutamic acid respectively. The mechanism by which sulphur becomes linked with carbon in compounds such as cystine has not yet been elucidated. Certain primary anabolites may undergo *sulphidation* or *sulphydrylation* in the presence of inorganic sulphides or hydrosulphides produced by the reduction of sulphates, which are absorbed by growing plants.

(ii.) *Ring formation.* The wide distribution of homocyclic and heterocyclic compounds suggests that living cells can unite atoms

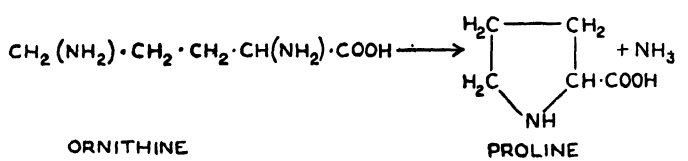
so as to form rings, or produce substances which condense spontaneously into cyclic compounds under the conditions prevalent in the cell sap. Purely chemical reactions that occur by the direct union of molecules must be distinguished from biochemical reactions, which only take place when protoplasm, or one of its enzymic components, has activated one or all of the reacting molecules. Cellular reactions that are purely chemical are nearly always preceded by preparatory biochemical reactions. The formation of the benzene ring from aliphatic compounds is probably a metabolic act. Various suggestions have been made concerning the possible modes of formation of phenolic and other benzene derivatives from primary anabolites containing six, three, or two carbon atoms, but no experimental evidence has as yet been obtained in support of these suggestions. It should be noted, therefore, that we are completely ignorant concerning the method by which this fundamental biochemical synthesis, on which the coal-tar industry is based, is effected by existing green plants and was effected in the carboniferous forests. Examples are given below of ring formation in aliphatic compounds, or in the side chains of cyclic compounds, which results from *internal condensation*, following molecular rearrangement (*a*) and (*d*), or the liberation of water (*b*), or ammonia (*c*).

(*a*) The formation of heterocyclic rings in pyranose sugars is spontaneous, *i.e.*, is independent of the presence of protoplasm. It should be noticed that the pyran ring is also represented in flavonic and anthocyanin pigments, and in catechin compounds. This does not necessarily mean that hexose sugars directly participate in the formation of these substances (*cf.* p. 185).

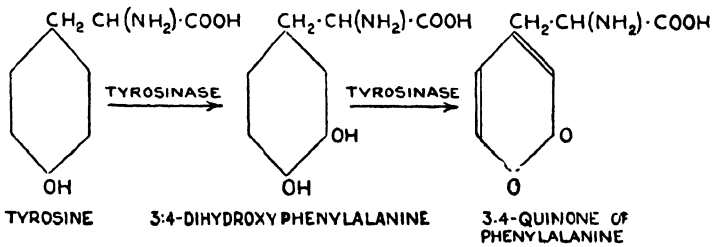
(*b*) Internal condensation may occur spontaneously with the elimination of water, as happens, for example, in the formation of orthocoumarin from the orthocoumaric acid set free by the hydrolysis of the coumarin glucosides of sweet vernal grass, sweet woodruff, and the tonka bean.

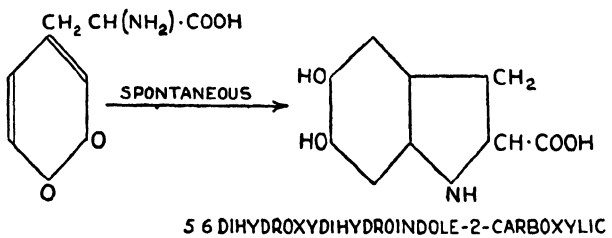


(*c*) Certain heterocyclic rings may arise from the internal condensation of aliphatic amino compounds (*e.g.*, amino acids such as ornithine and lysine), and ammonia may be liberated.



(*d*) Raper (*243*) has shown that melanin, which is formed from tyrosine by the action of tyrosinase, is a derivative of substituted indole. Evidently ring formation by the condensation of nitrogen and carbon must occur at some stage in the change, and, according to Raper, is possibly a spontaneous chemical event which follows the production of the reactive 3 : 4-quinone of phenylalanine from the enzymic oxidation by dehydrogenation of 3 : 4-dihydroxyphenylalanine. It will be observed that in the formation of this compound from tyrosine a hydroxyl group is introduced into the benzene ring. This is the only instance we can recall of the hydroxylation of a benzene derivative under the agency of an enzyme.





It should be noted that several further enzymic and other changes must take place before melanin is formed.

Ring formation may also be induced by *external condensation, i.e.*, by the fusion of two or more molecules of the same or of different substances, and doubtless occurs in living cells, but has never been experimentally achieved. Thus two unsaturated molecules may condense by means of one of the double bonds and give rise by addition to a cyclic compound, or condensation may occur with the elimination of water, ammonia, or other compounds. For example, it has been suggested that iminazole, pyrimidine, and purine rings may arise as a result of the condensation with dehydration of urea and methyl glyoxal (see Onslow, *214*, p. 238).

Possible relations between certain anabolic end products and secondary anabolites. One may regard such secondary anabolites as are found in the free state in cells, either as products of hydrolysis

of anabolic end products which contained glycosidal, ester, peptide, depside, or other linkages, or as the building stones for the synthesis of substances containing these linkages.

(i.) *The synthesis of anabolic end products containing one or more glycosidal linkages.* This big and varied class of end products (table VII) is comprised of polysaccharides (starch, cellulose, hemicelluloses, mucilages, and pentosans) and substances with polysaccharide affinities (pectins and gums); tetra-, tri-, and disaccharides; and the substances that have been grouped together as glycosides (p. 433). They are all hydrolyzed *in vitro* by acids, cleavage occurring at the glycosidal linkages (pp. 430 and 434), but not all by the living cells of green plants. Thus whereas starch, hemicellulose, inulin, and cane sugar—the carbohydrate food reserves of plants—are hydrolyzed by amylase, cytase, inulase, and invertase, respectively, enzymes that can hydrolyze cellulose, pentosans, mucilages, and gums, have not yet been separated from green plants, nor is there substantial evidence that these anabolic end products are used during growth. It is well established that during autolysis α-glucosides are hydrolyzed by maltase, and β-glucosides (*e.g.*, amygdalin) by the prunase component of emulsin (p. 26), but little is known concerning the fate of these substances in normal metabolism.

TABLE VII

Primary anabolites.	Anabolic end products containing glycosidal linkages.
Glucose	Glucosans (starch, cellulose, hemicelluloses, mucilages).
Fructose	Fructosans (inulin).
Glucose + fructose . .	Cane sugar.
Galactose + glucose + fructose.	Raffinose.
Mannose	Mannans (hemicelluloses, mucilages).
Galactose	Galactans (hemicelluloses, mucilages, pectins, gums).
Arabinose . . .	Arabans (hemicelluloses, mucilages, gums (*e.g.*, gum arabic), pectin).
Xylose	Xylans (hemicelluloses, gums (*e.g.*, wood gum), mucilages).
Monosaccharides + another substance (not a sugar).	Glycosides.

There is evidence in favour of the view that one must attribute the formation of some of the carbohydrate end products (*e.g.*, cellulose, inulin, and cane sugar) to the power which protoplasm possesses of synthesizing glycosidal linkages by combining, with dehydration, molecules of (i.) the naturally occurring monosaccharides, *i.e.*, glucose and fructose, or (ii.) monosaccharides and phenolic or other substances. Enzymic synthesis of glycosides has been effected (p. 28), and has led to the suggestion that in the carbohydrate group

also, synthesis may be attributed to hydrolytic enzymes when these are acting under favourable conditions in living cells. Evidently (see above) the synthesis of cellulose pentosans, mucilages, and gums, cannot yet be attributed to enzymes.

The hypothesis that starch is synthesized by maltase and amylase, acting synthetically on sugars in plastids, has never been experimentally verified. It cannot yet be said that this hypothesis is discredited. Hanes has, however, proved that starch may be synthesized *in vitro* when glucose-1-phosphate is acted on by phosphorylase (p. 63). The chemical change may be described as *dephosphorylative condensation.* It remains to be proved that the power to bring about this change is the only one that plastids use when they synthesize starch.

Attention is called to the fact that mannose, galactose, and pentoses, do not occur in the free state in living cells, but are widely distributed as mannans, galactans, and pentosans, in hemicelluloses, mucilages, etc. It may be that the monosaccharides undergo glycosidal condensation immediately they are formed. Alternatively, however, mannans and galactans might originate from glucosans or fructosans, and pentosans from uronic acid derivatives of polysaccharides. Some significance has been attached to the frequent association in plant cells of glucosans and xylans (*e.g.*, in wood gum), and of galactans and arabans (*e.g.*, in gum arabic and pectin), because glucose can by chemical means (oxidation and decarboxylation) be converted into xylose by way of glucuronic acid, and galactose into arabinose by way of galacturonic acid.

(ii.) *Anabolic products containing ester linkages.* Among plant products the ester linkage occurs in simple volatile esters (*e.g.*, amyl acetate), waxes, fatty oils, lecithins, pectin, and nucleic acid. The ester linkage readily undergoes hydrolytic cleavage *in vitro* in the presence of acids or alkalies, and enzymic cleavage of most of these compounds (but not of chlorophyll [1]) has been effected. Thus lipase (p. 25) hydrolyzes simple esters, fatty oils, and lecithins ; phosphatase hydrolyzes phosphoric esters, *e.g.*, lecithins and nucleic acid ; and pectinase hydrolyzes pectin to pectic acid and methyl alcohol. There is evidence that lipase and phosphatase can act synthetically, and it may be that in living cells the final stage in the synthesis of the anabolic end products named in table VIII is by ester formation from the corresponding secondary anabolites.

(iii.) *The synthesis of anabolic end products containing peptide linkages.* All proteins can be hydrolyzed by acids or by enzymes (protease and peptidases, p. 26), to amino acids by way of proteoses, peptones, and polypeptides (p. 447). It has been suggested that the

[1] The enzyme chlorophyllase (p. 25) effects the alcoholysis, not the hydrolysis of chlorophyll.

reverse process can occur in living cells. This would imply that protoplasm possesses the power of *producing peptide linkages*, and thereby of synthesizing proteins from secondary anabolites, viz., amino acids.

TABLE VIII

Secondary anabolites.	Anabolic end products containing ester linkages.
Lower aliphatic acid + lower alcohol . .	Volatile esters.
Higher aliphatic acid + higher alcohol . .	Waxes.
Glycerol + fatty acid	Fats.
Glycerol + fatty acid + phosphoric acid + choline	Lecithins.
Phosphoric acid + nucleoside	Nucleic acid.
Pectic acid + methyl alcohol	Pectin.
Chlorophyllins *a* and *b* + methyl alcohol + phytol	Chlorophylls *a* and *b*.

(iv.) *The synthesis of anabolic end products containing depside or other linkages.* The formation of other complex anabolic end products appears to be the result either of the condensation with dehydration or of the polymerization of secondary anabolites, such as phenols, phenolic acids or alcohols, fatty acids, and terpenes. For instance, in the production of tannins, molecules of phenolic acids (*e.g.*, gallic acid or protocatechuic acid) combine with loss of water to form didepsides, tridepsides, etc., and the resulting complex may then form a glycoside or ester with a monosaccharide. Lignin may be formed by the condensation with dehydration of phenolic alcohols (*e.g.*, hydrated caffeic alcohol). If this is the course of events one must assign to protoplasm the power of forming ether linkages (p. 44). Cutin and suberin result from the condensation and oxidation of higher fatty acids, while certain resins may be similarly formed from terpenes, and other resins by the condensation of phenols.

CHAPTER XII

THE EXPERIMENTAL STUDY OF METABOLISM

A. The Problem of Intermediate Metabolism Restated

ANABOLISM and catabolism are gradual processes. Probably there are many more intermediate stages in the synthesis of anabolic end products than we recognized in the last chapter, and there is little doubt that many intermediate substances have at least a transient existence in the hydrolysis of insoluble food substances and in respiratory oxidations. The ultimate objectives of the experimental study of intermediate metabolism are (*a*) to ascertain what substances accumulate and what transitory substances are produced during the metabolic transformations of known initial metabolites (A_1, A_2, A_3, say) to known metabolic end products (Z_1, Z_2, Z_3, say), (*b*) to place these intermediate metabolites (B's to Y's inclusive) in their proper sequence, (*c*) to discern the types of chemical reaction by which protoplasm effects each change in the sequence, and (*d*) to separate from protoplasm enzymic systems (e_a, e_b, e_c, etc.) that will effect each change *in vitro*. Evidently on attaining all these objectives we could give a complete description of the metabolic transformation in chemical terms.

The facts reported in chap. III indicate that for a number of catabolic reaction chains (*e.g.*, alcoholic fermentation) and for a few anabolic chains (*e.g.*, the phosphorylative synthesis of starch from sugar) the nature of the connecting links is known. For any substance (*e.g.* asparagine) we wish to know what is its precursor, and how in its turn this is produced, and what is its metabolic fate, anabolic and catabolic, in the living cell which makes it, or in some other cell to which it migrates. Attention is thus focused on the biological questions of location and function (pp. 172–180). To assign to purpose the cause of an event is to offer a teleological explanation of the event. This is not the explanation for which a biochemist seeks. To him function rendered possible is not a cause (see also p. 344). A substance is produced because molecules of substrates and enzymes, with possibly their coenzymes or other activators, meet in a certain way in a certain place at a certain time. What these molecules are, and how they react with one another, are the difficult problems which he has set himself to solve. Function is exercised because of the possession by the reaction products (*e.g.*, cellulose) of a certain molecular form (sometimes revealed with the aid of X-ray analysis), which determines its chemical and physical properties.

Chemists have not been backward in suggesting from purely

chemical considerations possible sequences of metabolic events. But it must always be borne in mind that a metabolic product may owe its origin *in vivo* to very different reactions from those by which it can be prepared *in vitro*. Accordingly, in order to test the worth of a hypothesis that has been based on knowledge of the chemistry of substrates participating in a metabolic change, data must be gathered by experimenting on living plants. If one definitely contradictory fact is experimentally established, a hypothesis must be rejected however attractive it may be from the chemical viewpoint. In the present chapter we are concerned with hypotheses which have been developed from broad biochemical studies on plants (section B) and with experimental methods which are available for testing in detail such hypotheses and the views put forward by chemists (section C).

B. Changes in the Chemical Composition of Plant Tissues under Natural Conditions

We shall consider below some of the more important views concerning metabolic sequences that have developed from the results of investigations on the chemical changes that accompany growth and differentiation, maturation, senescence, etc., and on the changes that result from natural alterations of external conditions, *e.g.*, of light intensity or temperature.

The metabolism of green shoots. We pointed out in the last chapter that the results of water culture experiments compel us to ascribe remarkable powers of synthesis to green leaves. We shall in a later chapter discuss the process of photosynthesis. In the present subsection we shall pass in brief review the evidence that, as a result of photosynthesis, green leaves are furnished with a rich supply of hexose sugars and that most of the subsequent metabolic transformations occur independently of the presence of light. Evidently it follows that such transformations may also occur in non-green parts of plants to which sugars are translocated. Green leaves must not be regarded as the only chemical manufactories in plants. Indeed it is probable that if they are supplied with sugars, mineral salts, and water, all living plant cells, in addition to consuming organic substances in respiration, can display very varied anabolic powers (see chap. XI, section F). The results of experiments point to the conclusion that in a given cell the concentration of sugars is, as a rule, a dominating factor in determining the extent and course of anabolic events. Accordingly, one may infer that in addition to photosynthesizing carbohydrates that are sooner or later translocated, green leaves play an exceedingly important part in the total metabolism of plants; for, evidently, while photosynthesis is in progress, a high level of concentration of sugars will be maintained in green leaves, and anabolism *in situ* promoted in many directions. Concen-

tration heads will be set up for such nitrogenous and non-nitrogenous compounds as are synthesized from carbohydrates, mineral salts, and water. The illuminated green leaves will consequently act as a source of nutrient substances, and the growing, storage, and certain other regions, as the sinks (see p. 137). Thus, as a result of translocation, the compounds formed in green leaves become available as substrates for metabolism in all parts of plants. Apart then from performing the essential operation of photosynthesis, green leaves may assist to a considerable degree in bringing about such syntheses of anabolic end products as accompany cell division and differentiation, and as lead to the accumulation of food substances in storage organs.

(i.) *The production of carbohydrates in green leaves.* Much discussion has centred on the results obtained by measuring the diurnal changes in the concentrations of reducing sugars (*i.e.*, *d*-glucose and *d*-fructose), cane sugar, and starch, in the green leaves of different plants.[1] For such leaves as are capable of producing starch, it can easily be shown that the amount of this substance contained in the chloroplasts increases during the day, and diminishes during the night (p. 225). Following the pioneer work of Brown and Morris on the leaves of Tropœolum, other investigators have made quantitative experiments on the sugars in leaves. Parkin experimented on snowdrop; Campbell on mangold; Davis, Daish, and Sawyer, on mangold and potato; and Miller on maize and sorghum. All the experimental results (*e.g.*, see those graphically recorded in fig. 23) indicate that during a 24-hour period the concentration of cane sugar may fluctuate considerably, increasing in the light, and decreasing at night (*cf.* the changes in the amount of starch), while the concentration of reducing sugars does not fluctuate in a manner that can be significantly correlated with light intensity.

The earlier workers interpreted these results as showing (*a*) that cane sugar is the first carbohydrate to be formed in photosynthesis; (*b*) that hexoses are derived from sucrose by hydrolysis; and (*c*) that starch often, but not always, accumulates as a secondary product of photosynthesis, and results from the condensation of sugars. They thought that the hexoses were translocated from the leaves, and, in addition, were used by the leaves in growth and respiration. But one can with equal justification infer from these results that hexoses are primary products, and that cane sugar and starch are secondary products of photosynthesis.

At the present day this view is widely held, and has received some experimental support (p. 219). It is now supposed that living cells cannot synthesize cane sugar or starch until, as a result of photosynthesis, a certain minimal critical concentration of hexose has been attained. The further production of hexoses above this critical

[1] See, *e.g.*, Onslow (*214*), Stiles (*287*).

amount is then immediately followed by condensations that lead to the synthesis of cane sugar and starch. Consequently the concentration of hexose remains approximately constant, while the concentrations of cane sugar and starch vary directly with the rate of photosynthesis. It still remains to be determined whether sugars must always be converted into glucose-1-phosphate before starch can be formed (p. 63).

The value of this concentration varies from species to species (see p. 218). In certain leaves, for example those of many monocotyledons, the critical concentration for starch formation is so high that

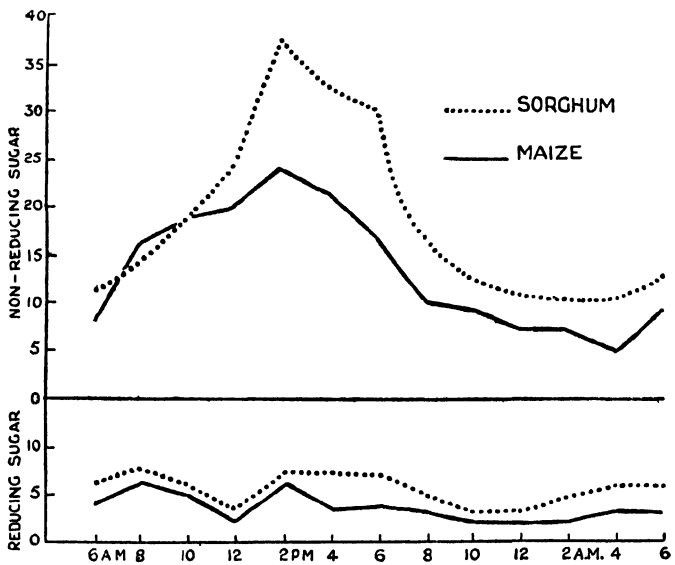


FIG. 23. Diurnal variations in the concentrations of reducing and non-reducing sugars in the leaves of maize and sorghum expressed in grams per square metre of leaf surface. (From Miller, *202*.)

starch never accumulates in these leaves under natural conditions. Cane sugar is, however, always produced. It must be remembered, however, that polysaccharides other than starch may be synthesized in such leaves. For example, fructosans have been detected in the leaves of wheat and barley (see Barnell, *15*; Yemm, *337*). It is of interest to note that in the summer of 1933, Yemm found starch in barley leaves picked in the late afternoon, but not in 1932, a less favourable season for the growth of plants. Possibly on the days he made his observations in 1932, photosynthesis had not been sufficiently active for the attainment of the critical concentration of sugar for starch formation.

In explaining the variations experimentally observed in the concentrations of carbohydrates we must remember that throughout the day and night sugars diffuse away from the leaves, and, in addition, continually undergo metabolism in the green cells in which they are produced. Under favourable conditions for photosynthesis the rate of production of hexoses exceeds that of the removal of carbohydrates, and, consequently, cane sugar and starch accumulate as secondary products in the mesophyll tissue. When the light fails, photosynthesis ceases; but translocation and respiration, and other types of metabolic change, continue, and the chemical equilibria characteristic of the cells are maintained by the conversion into hexoses of some or all of the cane sugar and starch stored during the day. As a result the concentration of each of these condensates falls.

(ii.) *The protein metabolism of green leaves.*[1] It has been found that under natural conditions (*a*) the concentrations of protein and of total nitrogen in a green leaf may increase during the day, while the concentration of nitrate is always extremely low; (*b*) the concentrations of protein and non-protein nitrogen decrease during the night, while the concentration of nitrate increases, and (*c*) in illuminated variegated leaves there may be considerable amounts of nitrate in the white parts, but none at all in the green. Considering this evidence alone, one might infer that protein is synthesized either from nitrate and the carbohydrates that are produced by photosynthesis, or direct from carbon dioxide, water, and nitrate, by a special form of photosynthesis in which carbohydrates are not produced. These alternative hypotheses have been tested by feeding detached leaves placed in the dark with sugars and with nitrates or ammonium salts. Evidence has been obtained that under these conditions proteins may be synthesized, provided the concentration of sugar is sufficiently high (*cf.* starch formation by feeding darkened leaves with sugar). Accordingly, the inference has been made that light does not participate directly in the synthesis of proteins by green leaves.

Moreover, it has long been realized that the successful growth of saprophytic bacteria and fungi (*e.g.*, yeast) in nutrient solutions containing sugars or some other nitrogen-free organic compounds, ammonium salts, and certain other mineral salts, shows that nitrogen can enter into organic combination independently of the presence of light. The successful culture of roots in solutions of similar composition provides further evidence of this (p. 205). It will be recalled that growth implies the synthesis of the constituents of protoplasm.

Among the important feeding experiments carried out in recent years are those of Pearsall and Billimoria (*226*). They selected daffodil leaves for study, because the surfaces of these leaves can be

[1] Chibnall (*59*) has written an important monograph on this subject. Earlier reviews by Onslow (*214*) and McKee (*197*) contain suggestive discussions.

sterilized without injury, by washing them first with a solution of calcium hypochlorite and then with sterile water. The sterilized leaves were divided into four segments, white meristematic base, yellow-green extending zone, lower green photosynthetic zone, and green apical region. They then studied the synthetic powers shown by these regions when the segments were floated on an 0·1 molar phosphate buffer solution at *pH* 6, containing three per cent. glucose and 0·3 per cent. ammonium nitrate. The rate of air supply to the cells appears to have been adequate, and, presumably, there was sufficient sulphate in the cell sap to meet the sulphur requirement in protein synthesis. From their results they constructed balance sheets that showed the fate of nitrogen in a closed system, which remained free from bacteria during the experimental period of sixty hours. All the segments absorbed ammonium nitrate and converted it into nitrogenous organic compounds (but not necessarily into protein), *i.e.*, each segment of the leaf could bring about that all-important biochemical event, the union of carbon and nitrogen atoms. It was found that light usually accelerated this production of organic nitrogen in the pigmented segments, but not in the basal ones. Pearsall and Billimoria assert that " this effect of light has previously evaded reasonable proof." They obtained no evidence of protein synthesis in the green zones, but proteins were synthesized actively in the meristematic regions, and to a less extent in the yellow-green extending zones. It is important to note that light had no effect on the rate of protein synthesis in the basal zone, for this means that the synthetic powers of leaf meristems may resemble those of root meristems. Only in the yellow-green zones did illumination bring about notable net gains in the protein content. Pearsall and Billimoria consider that as leaves turn green and become " young-mature " they lose the power of synthesizing proteins under the conditions of their experiments (*i.e.*, the possibility of synthesis by such leaves when attached to a plant is not denied), but retain the power of synthesizing other and simpler nitrogenous compounds. Since light accelerates the latter synthesis in yellow-green tissue, there will occur in such tissue a more rapid conversion of inorganic nitrogen to organic nitrogen, and consequently a more rapid production of protein. During the course of their experiments these investigators discovered that small amounts (up to 20 mg. per 10 gm. of leaf per sixty hours) of free nitrogen might escape from the system as a gas while nitrate was being converted into amino compounds. There is good evidence that this conversion occurs in the sequence nitrate → nitrite → ammonium salts → amino compound. They (225) think it is probable that the loss of free nitrogen results from a reaction between nitrite and amino compounds.

Although the recently obtained evidence we have discussed

suggests that light may under certain conditions promote the synthesis by green leaves of nitrogenous organic compounds from sugars and inorganic salts, it appears to be well established that, in leaves well supplied with carbohydrates and salts, such compounds (including proteins) may also be synthesized in the absence of light. We must, of course, remember that light is essential for the production by green plants of proteins from carbon dioxide, water, and inorganic salts, since it is required for the photosynthesis of sugars. Clearly, therefore, the rate of photosynthesis will have a determining influence on the rate of protein synthesis, not only in green leaves, but in all parts of a plant. The metabolic sequences in such syntheses of nitrogenous organic compounds as occur in green leaves may well be similar to those taking place in non-green cells (*e.g.*, those of meristematic or storage regions) that show active nitrogen anabolism in other parts of a plant, not only in the production of proteins from amino acids, but also in changing one amino acid into another (see p. 204), and in synthesizing amino acids from inorganic nitrogen and sugars.

Clearly the fundamental question is this *synthesis of amino acids*. Knowledge about it has increased in recent years ; indeed, an enzymic synthesis *in vitro* has been achieved (p. 48). In 1925 Knoop and Oesterlin suggested that α-keto acids might react with ammonia to produce α-imino acids, which would, by reduction, yield α-amino acids. This theory has received support, from the results of feeding experiments on surviving animal tissues. In von Euler's school at Stockholm evidence has been obtained that, under the agency of an animal enyzme, glutamic acid may be synthesized by this mechanism from ammonia and α-ketoglutaric acid. We are beginning to see possible ways by which primary organic anabolites, derived from carbohydrates and such ammonium salts as are produced by the reduction of nitrates, may participate in that all-important step in plant metabolism, (and, therefore, in preparing for the nutrition of animals), the synthesis of an amino acid (see also pp. 46 and 187). The analyses of leaf proteins, carried out in Chibnall's school, show that green leaves can synthesize nearly all the known amino acids. Inasmuch as there is much variety in the structure of their molecules (p. 446), it will be a big task to elucidate the nature of all the biochemical mechanisms which are brought into action in synthesizing them.

Under *cell conditions favouring condensation* as well as amino acid synthesis, *e.g.*, those prevalent in young illuminated leaves (see Pearsall and Billimoria, p. 200 above), *proteins are synthesized from amino acids*, and there may also be formed amides and other nitrogenous compounds (*e.g.*, chlorophyll). We may postulate the existence of *a reversible equilibrium between plastic proteins and their*

degradation products, among which we include amino acids and ammonium salts. This equilibrium may be compared to the one between starch and sugar. When synthesis is active a diffusion head will be set up. *Crystalloid nitrogen, possibly as residual nitrogen* (p. 146), *will be translocated in the phloem* from the mesophyll of attached leaves to growing and storage regions of shoot and root. Returning to the consideration of the experimental results (*a*) and (*b*) recorded on p. 199, we see that the synthesis of organic nitrogen may more than compensate for loss of nitrogen by translocation. This would account for the synthesis and temporary storage in mesophyll of protein (*cf.* the parallel phenomenon of the accumulation of starch). In the dark translocation continues, but the synthesis of nitrogenous organic compounds is greatly retarded or may stop. The migration from leaves of amino acids will lead to the hydrolysis of storage protein (*cf.* the disappearance of starch from darkened leaves). Accordingly, the amounts of protein and of total organic nitrogen in an attached leaf will decrease in the dark.

Chibnall (*59*) has put into good order the confused evidence about the conditions governing *the production of amides* (*asparagine and glutamine*) *and ammonium salts by green leaves and other organs*, and about their functions. The following statements will indicate the nature of some of the ground covered by investigators in this field. Asparagine is readily produced in some families (*e.g.*, Leguminosae), but less readily in others (*e.g.*, Cruciferae). In these, however, glutamine may accumulate. According to Chibnall, Schwab's experiments " leave no doubt that in most families, both amides may be elaborated and in proportions which vary in the different regions and with the stage of development of the plant." Ruhland and Wetzel drew a distinction between *amide plants* and *acid or ammonium plants*. In the latter (*e.g.*, Begonia, whose leaves have a *pH* value of 1·5) ammonium salts of vegetable acids (*e.g.*, malic and oxalic acids) are produced, especially in the dark, and, so they maintained, to the exclusion of amides. More recent work has, however, shown that acid or ammonium plants can also produce amides. For example, during five days in the dark, Oxalis leaves, whose sap had a *pH* value of 1·5, lost 55 units of protein nitrogen whilst accumulating 27 units of ammonium nitrogen and 17 units of glutamine nitrogen. Pelargonium leaves, whose sap had a *pH* value of 2·8, lost 43 units of protein nitrogen whilst accumulating 27 units of ammonium nitrogen and 8 units of asparagine nitrogen. The nitrogen unit here is the percentage of the total nitrogen in the leaf. It will be observed that not all the loss of protein nitrogen was accounted for by the ammonium and amide nitrogen which accumulated. Some of it may have remained as amino nitrogen in the amino acids formed by the hydrolysis of proteins, which would be the first step in their catabolism. Yemm

(p. 274) found that glutamine and asparagine began to accumulate in detached barley leaves, which are not acid leaves, during the first day of starvation in the dark. The concentration of ammonia was rising steeply after two days. In none of these experiments were the conditions natural, but the results seem to suggest that certain enzymes (*e.g.*, proteases, amino acid dehydrases, amidases) may be present in the leaves of all plants, whether they are amide plants or acid or ammonium plants. Accordingly, protein catabolism in starving leaves of all kinds may lead to the accumulation of amides, which are not toxic, and free ammonia, which is a poison.

When the effects of protein catabolism on the health of plant cells is considered, it may possibly be better to classify plants as *acid and non-acid*, since both kinds may be amide plants. Ruhland and Wetzel suggested that, in the former, toxic ammonia is rendered innocuous by combining with vegetable acids. But such ammonia as is not so changed quickly kills cells in non-acid plants (see Yemm's experiment p. 274). In neither of these two kinds of plants will ammonia, produced in protein catabolism, do damage, if it immediately becomes incorporated in newly formed amino acids or in an amide.

When it is stated that amides are produced during the catabolism of proteins, it does not mean that the non-nitrogenous parts of all the molecules of glutamine and asparagine have been exclusively derived from amino acids set free by the action of proteases. When there is no external supply of nitrogen the NH_2 — in the amino and amide groupings will, of course, come from protein nitrogen. Very often the amounts of glutamine and asparagine accumulating are much in excess of the amounts that can be accounted for by the quantities of glutamic acid and aspartic acid set free by protein hydrolysis. The modern view is that amides are synthesized from α-keto acids by the reactions briefly described on pages 27, 48 and 69; asparagine finally coming from the union of oxalacetic acid and ammonia, and glutamine from that of α-ketoglutaric acid and ammonia (see also fig. 1). The origin of these α-keto acids is, clearly, a fundamental problem. Mechanisms are known by which they can be produced from amino acids, and, therefore, by the catabolism of proteins. Chibnall, however, cites evidence for the view that by far the larger part, if not all, of the non-nitrogenous structures in amide molecules are derived from carbohydrates, and points out that it takes a long time, even under starvation conditions, to deprive leaves completely of their carbohydrates. Reactions by which oxalacetic acid and α-ketoglutaric acid may possibly be produced from sugars are included in those set out in fig. 1, and are discussed in chap. XV.

When amides are produced under natural conditions in illuminated green shoots of entire plants, the nitrogen used may be absorbed from

the soil, or it may come from protein, manufactured in a green leaf or stored in a seed or other perennating organ. The experimental evidence suggests that when there is an ample supply of nitrogen and no shortage of sugar, *i.e.*, when the green shoots are well illuminated, amide formation is favoured. A striking experiment was performed by Greenhill and Chibnall, who found that so much glutamine was produced by perennial rye grass, heavily manured under natural conditions with ammonium sulphate, that this amide exuded from and crystallized on the surfaces of the shoots. Chibnall pictured a chemical mechanism in which ammonia combines with α-ketoglutaric acid derived from sugar in the leaves of this grass. Using an injection method he obtained evidence of the existence of such a mechanism when he showed that glutamine formation is promoted by feeding leaf cells with ammonium α-ketoglutarate.

In the mesophyll cells of leaves growing under natural conditions and not supplied from outside with excessive amounts of ammonium salts, the concentration of amides is usually low. In the cotton plant there is a negative concentration gradient from the leaves downwards. ' Mason and Maskell (p. 146) concluded that asparagine is a storage product, synthesized in the phloem from translocatory nitrogen. Presumably carbohydrates may participate in this synthesis. To decide whether this conclusion is generally valid, we require information about the distribution of amides in classical objects of study, *e.g.*, germinating leguminous seeds. It is possible that amide formation in the phloem is a normal event in the internal economy of plants, whereas in leaves it may be an accident attaching to abnormal conditions. Since there is no reason to suppose that the biochemical mechanism requisite for amide formation is different in different parts of plants, researches on oxidative amination, transamination, amidation, and the like, carried out on leaves, where these reactions may not normally occur to any great extent, will illuminate similar events taking place elsewhere, which are of real functional significance, but which may be more difficult to investigate.

Chibnall has made a suggestion that may turn out to be of outstanding importance. It is that one of the essential functions of amides is their power of acting as regulators in the synthesis of specific proteins in growing and storage tissues. Here is a quotation from his book. " The proteins of the growing parts may have a different amino acid composition from those of the reserve organs. Protein regeneration, therefore, in the absence of external sources of nitrogen, may necessitate an interconversion of amino acids . . . and this probably takes place through the agency of asparagine and glutamine." The action of some enzymes that may be concerned in such transformations has already been described (p. 49).

(iii.) *The production by shoots of vegetable acids.* The evidence which indicates that carbohydrates manufactured by green leaves may be converted into vegetable acids is presented in chap. XV.

(iv.) *The production of aromatic compounds and other substances in green leaves.* Experiments have shown that tannins, anthocyanins and other glycosides, and many other types of substance, may accumulate in illuminated green cells. It is reasonable to suppose that most of these substances result from the metabolism of carbohydrates produced in photosynthesis, and are not themselves photobiochemical products. The production of chlorophylls *a* and *b*, however, is, as a rule, dependent upon the presence of light as well as upon a supply of carbohydrates.[1] Quantitative data showing relations between concentrations of sugars and of other components are not numerous; but we note that Barnell (*15*), in his studies on the seasonal changes in the carbohydrates produced by shoots of wheat, found that the drifts of the concentrations of free glucose and of glucosides were closely similar.

The metabolism of roots and excised embryos growing in culture solutions. Inasmuch as a vast amount of biochemical knowledge has been gained by quantitative studies on bacteria, yeast, and mould fungi, growing in culture solutions of known composition, attention is drawn to the biochemical importance of the discovery that roots grow and develop when root tips are sown in culture solutions containing sugar, inorganic salts, and the proper hormones (p. 383), and that, under similar conditions, seedlings have been produced from excised embryos. We may immediately infer that the cells of the root tips and embryos possess the power of synthesizing from the simple substances present in the culture solution, all the exceedingly complex substances as well as the simple substances found in protoplasm, cell walls and vacuoles, *i.e.*, nearly all the substances described in Appendix I. Clearly the methods which have been used in studying the metabolism of growing yeast may well prove productive when applied to the study of roots and whole embryos growing in culture solutions.

The metabolism of germinating seeds. The determination of the changes in chemical composition that take place during the germination of seeds has thrown light on the catabolic events that lead to the production of respiratory energy and to the mobilization of reserve foods, and has also provided direct evidence concerning the chemistry of some of the anabolic events associated with growth. The results of such experiments show the typical chemical relations that hold between the metabolism of regions that supply foods (green leaves; the storage regions of rhizomes, bulbs, corms, tubers, etc.; storage parenchyma in woody perennials) and that of regions in which food is

[1] See review by Priestley (*234*) of Lubimenko's experiments.

assimilated for growth (sprouting buds, growing apices of shoots and roots, and cambial regions).

In table IX are recorded the results of analyses of comparable samples of seeds and seedlings. The loss in dry weight may be attributed to respiration, and this process will account for about one-third of the observed decrease in the fat content. The respiratory quotient was about 0·7. The remainder of the decrease may be ascribed to the conversion of fats into carbohydrates, lecithin, and, possibly, soluble organic acids. Although finely emulsified fats can migrate from storage tissues, it is fairly certain that the mobilization of fats in the cotyledons was mainly brought about by lipase cleavage and by the conversion of the glycerol and fatty acids thus produced into diffusible carbohydrates. Glycerol has never been detected in germinating seeds. Consequently one may conclude that it is rapidly

TABLE IX. *Chemical changes during the germination of sunflower (after Frankfurt, see Palladin, 223)*

	Seeds.	Seedlings.	Gain or loss during germination.
Total dry weight .	100	88·98	− 11·12
Simple proteins . .	24·06	13·34	− 10·72
Nuclein and plastin [1] .	0·96	4·05	+ 3·09
Asparagine and glutamin	0·00	3·60	+ 3·60
Lecithin . . .	0·44	0·71	+ 0·27
Fats . . .	55·32	21·81	− 33·50
Sugars . . .	3·78	13·12	+ 9·34
Soluble organic acids .	0·56	2·16	+ 1·60
Cellulose . . .	2·54	10·25	+ 7·71
Hemicelluloses [2] . .	0·00	3·41	+ 3·41

converted into carbohydrates (*cf.* p. 218). Since free fatty acids may accumulate, it appears that the rate of lipase cleavage may exceed that of the conversion of fatty acids into sugars. We may ascribe to cell wall formation in the growing apices the carbohydrate anabolism that led to increases in cellulose and hemicelluloses.[2] It will be noticed that the disappearance of reserve protein from the cotyledons may be accounted for by the synthesis of conjugate proteins in the growing apices. Mobilization of the protein was probably effected by hydrolysis under the agency of proteolytic enzymes (see pp. 26 and 27). Amino acids are not represented in the above table, but they were probably present. It is evident from the differences between gains and losses in weight that Frankfurt did not succeed in making a complete analysis. Amino acids have, however, often been shown to accumulate during germination. The demonstration of the production of amides is of considerable interest. The reader is

[1] Presumably nucleoprotein and lipoprotein.
[2] Pectic substances were probably estimated as hemicelluloses.

referred to the discussion on p. 203, for it is probable that protein catabolism in seeds is very similar to protein catabolism in darkened leaves, although it may be more intense. For example, high concentrations of asparagine may develop during the normal germination of leguminous seeds, which are rich in protein. A progressive accumulation of asparagine occurs if the germination of such seeds is brought about in the dark, in order to preclude the replenishment of carbohydrate stores by photosynthesis. If etiolated seedlings turn green and continue to grow upon exposing them to light, the asparagine gradually disappears. Good evidence exists that its nitrogen is used in protein synthesis. According to Chibnall the $-NH_2$ groupings in asparagine and glutamine have special functions when the amino acid composition of the protein synthesized in the meristems differs from that of the reserve protein in the storage tissue (p. 204). In the meristem not only will amino acids translocated from the storage tissue be directly used in the manufacture of new protoplasm, but an interconversion of amino acids must occur in building the specific proteins in the meristematic cells.

Seeds containing carbohydrates as the principal food reserves have also been chemically studied. Their protein metabolism is similar to that in the germination of seeds containing fatty oil as food reserve. Where starch is the reserve food (*e.g.*, in cereal grains or leguminous seeds), carbohydrate catabolism in the endosperm or cotyledons is similar to that in darkened green leaves containing starch. Amylase and maltase hydrolyze starch to glucose, which diffuses to the growing apices and is used for cell wall formation. Cytase hydrolyzes the hemicelluloses in date seeds and elsewhere, and converts this food reserve into diffusible sugars. Dry weight is lost as a result of the respiratory oxidation of carbohydrates, and for seeds belonging to this class the respiratory quotient is unity.

The results of quantitative experiments performed by Barnell (*16*) on the respiration of young barley seedlings show clearly the relations that exist between certain biochemical events occurring in different parts of germinating seeds. In the endosperm of swollen barley grains starch is hydrolyzed to sugars, but the storage tissue shows only feeble powers of respiration. It loses dry weight, however, owing to the translocation of sugars to the embryo, which consumes the sugars rapidly in growth and respiration. It should be noted that whereas loss in dry weight of the *whole* germinating grain results mainly from the respiration of the embryo, it is the endosperm which actually loses dry weight: the dry weight of the growing embryo increases. As Barnell points out, the functions of endosperm and embryo are linked through translocation, and under certain conditions the rate of translocation may determine the rate of occurrence of events in the embryo.

A. L. James (*148*) has reported that sucrose, possibly produced in the endosperm and translocated therefrom, plays a dominant part in the carbohydrate metabolism of the developing embryos of barley, providing substrate for respiration and for the formation of the normal cellulose and hemicellulose of which the cell walls are composed. Raffinose was present in the embryos of ungerminated grains, but quickly disappeared during germination in the dark. Over a period of five days the concentration of hexose hardly changed, but that of the fraction measured as maltose increased. James's report of the presence of maltose is noteworthy, as it has generally been supposed that this disaccharide does not accumulate in growing plants.

The metabolism of developing storage tissues and feeding tissues. Great practical importance attaches to the investigation of the chemical changes occurring during the development of seeds, tubers, swollen roots or hypocotyls, etc., and during the growth, ripening, and senescence of fleshy fruits. We only have room here to consider certain aspects of the metabolism of two types of tissue, viz., the developing storage tissues in seeds, and the flesh tissue of growing and fully-grown apples.

TABLE X. *Changes in carbohydrate and fat content of ripening almonds (from Leathes and Raper, 179)*

Date.	Oil. Per cent.	Sucrose. Per cent.	Glucose. Per cent.	Starch. Per cent.
June 9th . .	2	6·7	6·0	21·6
July 4th . .	10	4·9	4·2	14·1
August 1st . .	37	2·8	0·0	6·2
September 1st .	44	2·6	0·0	5·4
October 4th . .	46	2·5	0·0	5·3

(i.) *Developing storage tissues in seeds.* It has been found by means of chemical analysis and by applying microchemical tests, that the sugars translocated to maturing seeds are gradually converted into polysaccharides (starch or hemicellulose) or into fatty oils (table X). It is not always realized that the occurrence of fatty oil as a reserve food in seeds is far more general than that of starch. The chemical reactions which may possibly take place in the conversion of carbohydrates into fats are discussed on p. 188. It has been observed that fatty acids sometimes accumulate in the early stages, and later disappear.

Protein is produced in all seeds either (*a*) by the condensation of amino acids translocated from green leaves or elsewhere, or (*b*) by reactions in which carbohydrates, amides, and ammonium salts may participate. According to Chibnall (*59*) leaf and seed proteins of the vegetable pea have a somewhat similar amino acid composition. He concludes that in its developing cotyledons reaction (*a*) may predominate. Since, however, the

composition of gliadin and glutenin in the wheat grain differs so widely from that of the proteins which have been examined in the leaves of the Gramineae, he thinks that there are extensive interactions among translocated amino acids. A possible mechanism is described on p. 49. In addition amino acids may be synthesized from ammonia and α-ketoacids, derived by the cleavage of sugars (p. 201). Accordingly in the wheat grain reaction (*b*) as well as (*a*) may take place.

(ii.) *The metabolism of the flesh tissue of growing and fully grown apples.* Much information has been obtained concerning the physiology of the apple, as a result, in particular, of the investigations which have been promoted by the Food Investigation Board in this country,[1] and by Departments of Agriculture in the United States of America. All we can attempt here is a brief review of certain selected biochemical phenomena.

During the growth phase that immediately follows fertilization, cell division proceeds rapidly. The constituents of protoplasm (p. 8) and of cell walls (p. 172) are synthesized from the nutrient material translocated

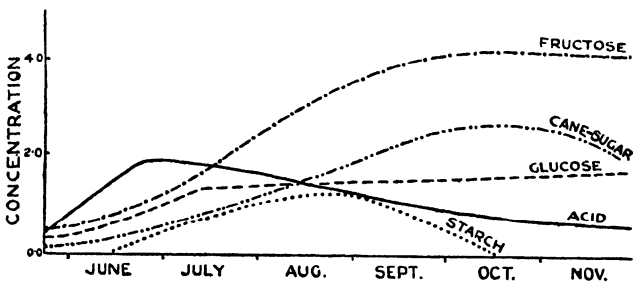


FIG. 24. Variations in the concentration of certain constituents of the flesh tissue of Bramley's seedling apples during growth. The results are expressed in grams of substance per 100 grams fresh weight of tissue. The apples were picked on October 21st. (From Archbold, *4*, modified.)

from green leaves and storage tissue. During the subsequent vacuolation and enlargement of cells, cell wall material continues to be laid down, and the concentrations of carbohydrates and organic acids in the flesh tissue gradually increase (fig. 24).

Franzen and Helwert (*92*) found considerable amounts of citric as well as malic acid in the variety of apple they analysed. Succinic and lactic acids were also present in small amounts. They detected traces of oxalic acid, and obtained evidence of the presence of unsaturated acids. Archbold's investigations show that the concentration of acid in the Bramley's Seedling apple reached its maximum by the middle of June, and then declined. The question of the origin and fate of organic acids in plants is discus sed in chap, XV.

Touching the fluctuation in the concentration of carbohydrates, we notice that Archbold found that the concentrations of monosaccharides and cane sugar steadily increased during the growing period, and that the concentration of monosaccharides was always greater than that of cane sugar. Kidd, however, has reported that the concentration of cane sugar may begin to

[1] See the annual reports published since 1919 by this board.

decrease in July (see fig. 26). If, as is now supposed (p. 145), cane sugar is translocated to the fruit from leaves or from storage tissue, monosaccharides found in the apple must be formed as a result of the hydrolysis of this sugar. It is an interesting fact that in Archbold's experiments, the sugar concentration had by June become sufficiently high to induce starch formation (*cf.* p. 198). The reason for the subsequent gradual increase in the starch concentration is readily understood, but it is difficult to account for the fall which began in August and led to the disappearance of starch in October, while the sugar concentration was still rising. In the experiments reported by Kidd, and in those more recently described by Kidd and West (*169*), the concentrations of cane sugar usually increased during the hydrolysis of starch, reached a maximum, and then gradually diminished. The apparent conversion of starch into cane sugar is a cellular event that is surprising from the chemical standpoint. It appears to occur in a number of other

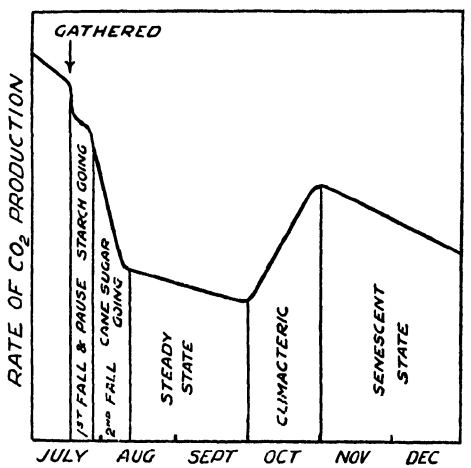


FIG. 25. Changes in the respiratory activity of the apple during growth, ripening, and senescence. (From Kidd, *165*.)

tissues (*e.g.*, potato, see p. 218). It should be noted that Kidd and West have detected a component other than starch in the alcohol-insoluble fraction of the carbohydrates in Bramley's Seedling. They state that the final analysis of carbohydrate transformations in the apple must await a further investigation of this fraction. An important point which they stress is the probable interconversion in the apple of fructose units, *i.e.*, free fructose or compounds (*e.g.*, cane sugar) containing fructose, and glucose units, *i.e.*, free glucose or compounds (*e.g.*, cane sugar, starch) containing glucose. Such interconversions have been demonstrated in other tissues (pp. 276–7).

Before considering the changes that occur in the ripening of an apple in the orchard or in a storage chamber, we must call attention to the fact that apple tissue is continually absorbing oxygen and giving off carbon dioxide. This respiration, of course, implies the continuous loss of carbohydrates (fig. 25). According to Kidd and West (*169*), who have performed extensive investigations on the respiration of the apple, all the fractions among carbohydrate components may be involved. They have also found (Kidd

and West, *168*) that the respiratory activity diminishes rapidly during the early summer and subsequently, during cell enlargement, more slowly. These decreases doubtless result mainly from the gradual increase in the water content of the fruit. For a period the respiration of the fully grown apple remains steady. Sooner or later, however, there is a sharp rise in respiratory activity, which continues until the fruit becomes green-yellow (Blackman and Parija, *32*). A new phase in the life of the apple has begun. Kidd and West have described this phase as the climacteric.

The charts drawn in figs. 25 and 26 indicate that the onset of the climacteric cannot be ascribed to an increase in the concentration of respirable sugars, although the enhanced respiration shown during this phase implies that sugar was consumed at a more rapid rate than in the less mature apples. Blackman has suggested that the rise in respiration in

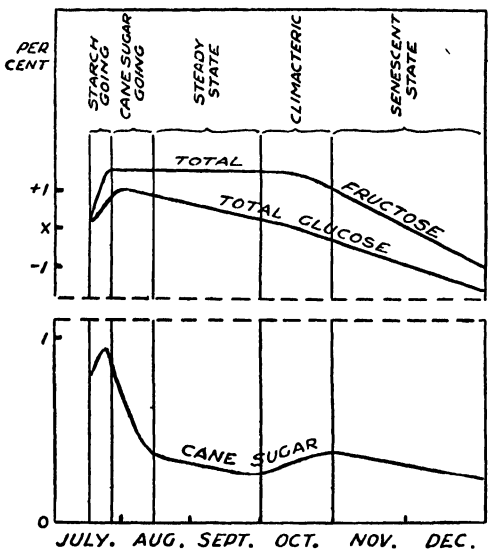


FIG. 26. Changes in the concentration of glucose, fructose, and cane-sugar, during growth, ripening, and senescence of the apple. (From Kidd, *165*.)

yellowing apples results from a decrease in organization resistance (p. 280). This decrease would lead to an increased rate of diffusion of molecules of respirable sugars to the respiratory centres in the protoplasm, and, consequently, to an enhanced respiratory activity. Clearly we must next consider the question of the possible cause of this decrease in organization resistance.

The answer now given to this question is based on the results of experiments which have shown that ethylene gas in very low concentration (say, one volume per million volumes of air) hastens the yellowing of citrus fruits and bananas, and affects in various ways the growth of plants (see, for example, Niederl *et al*, *210*, Huelin and Barker, *144*). For example, it retards the growth of pea epicotyls and causes swellings. Evidence was also obtained that ripe bananas give off a gas that accelerates the ripening of green bananas; the early ripening of a single bunch may hasten the ripening of all the bunches of bananas in a barrel. In 1932 Elmer reported

that a gas emanating from apples affects the sprouting of potatoes ; and, later, at the Low Temperature Research Station, Cambridge, it was shown that ethylene in low concentrations has the same effect. In 1934 Gane, using the well-known bromine test, detected ethylene among the volatile products given off by yellowing apples.

The influence of ethylene on respiratory activity has been investigated at Cambridge and elsewhere. Huelin and Barker (*loc. cit.*) have produced evidence, which supports conclusions advanced earlier by Herklots, that ethylene stimulates the respiration of such potatoes as have a low sugar content (see p. 280) by bringing about a decrease in organization resistance, *i.e.*, by advancing the rate of supply of sugars to the respiratory centres. Broadly the effect may be described as reversible ; when treatment with ethylene is stopped, respiration falls to its normal air value. Huelin and Barker report that other workers have found that the respiration of fruit in the preclimacteric phase is also stimulated by ethylene, but that this stimulating effect on fruit is irreversible. Moreover, after the onset of the climacteric, treatment with ethylene does not stimulate respiration.

Important inferences have been made from these experimentally determined facts. In the first place it has been argued that, as in the potato, so also in the apple, and indeed in other fruits (*e.g.*, banana, tomato), ethylene brings about a decrease in the organization resistance of the cells. At a certain stage of maturity fruits *by their own metabolism* produce ethylene ; and, accordingly, it is considered that the climacteric represents a state of autostimulation, in which yellowing and enhanced respiration are two of the attendant effects. There may be many others. The production of ethylene is continuous, which means that the passage through the climacteric phase, once it has started, cannot be stopped, although it may be retarded. Before the preclimacteric phase, fruit does not produce its own ethylene ; consequently, treatment of the green fruit with low concentrations of ethylene during this earlier phase hastens the onset of the climacteric ; but as soon as this stage is reached the fruit is in a state of autostimulation ; accordingly, the increase in the rate of respiration and of the rate of yellowing cannot be reversed by stopping the supply from the outside of ethylene. Evidently a good case has been made out for ascribing to the production and properties of ethylene, acting in low concentrations, the changes in cells that lead to and determine events during the climacteric period. There still remain for solution in the future the problems of finding out what is the source from which ethylene springs as a metabolic product, and of ascertaining what cell changes determine that it should be produced in fruits at a certain stage of maturity.

Metabolic events that lead to the production of odour and flavour are promoted during the climacteric phase. Power and Chestnut (*231*) detected acetaldehyde, and the methyl, ethyl, and amyl esters of formic, acetic, caproic, and caprylic acids, among the volatile products that developed during the ripening of certain American apples (*e.g.*, Ben Davies). They also obtained some evidence of the presence of the terpene, geraniol, or its esters.

The climacteric is followed by the phase of senescence. During this phase autolysis gradually sets in. Insoluble protopectin is converted by protopectinase into soluble pectin, and, with the disappearance of the middle lamella from cell walls, the flesh tissue may become mealy. The rate of CO_2 output declines during senescence, *i.e.*, when the fruit turns yellow, and afterwards. Kidd has represented this fact in the generalized curve reproduced in fig. 25. Since the respiratory quotient does not diminish—indeed it may increase (see p. 268)—it follows that oxygen

uptake is also depressed as the apple grows old. Fidler (*86*) found that ethyl alcohol and acetaldehyde steadily accumulate, and suggested that this phenomenon (senescence zymasis) might be attributed to the progressive retardation of oxidative processes. Thomas (*305*) had earlier demonstrated that these substances accumulate when the flesh tissue of an apple or a pear suffers injury (injury zymasis). On the basis of Kostytschew's views concerning respiratory events (p. 300), he suggested that during autolysis the intricate co-ordination of enzymes in the respiratory centres of the protoplasm breaks down, while the zymase system still retains its activity. Zymasis is a symptom of disorganization, but not the inducing cause. He found that 100 grams of apple tissue may at death contain more than 0·15 grams of alcohol. The apple belongs to the direct oxidase group of plants, and consequently turns brown on injury (p. 29). During browning oxygen uptake proceeds vigorously, and in consequence the apparent respiratory quotient falls rapidly (p. 268).

One of the chief objects of industrial research on the storage of fruits is to delay the onset of autolysis. Low-temperature storage, but well above the freezing point, is the chief requirement. Kidd and West have shown that this object may also be achieved by gas storage (see p. 155). The use of carbon dioxide and of atmospheres poor in oxygen depresses respiratory activity, retards ripening, and lengthens the storage life of a fleshy fruit. Extensive trials have been carried out in order to determine the behaviour of different varieties in gas storage, and to ascertain the optimum conditions for gas storage. For example, it has been found that Bramley's Seedling stores better at 5° Centigrade than at 1° Centigrade (see Kidd and West, *169*).

C. Special Biochemical Methods for Testing Hypotheses concerning Metabolic Sequences

In order to assess the value of the various hypotheses concerning metabolic sequences put forward from purely chemical considerations, or developed from general physiological studies such as those discussed in section B, experimental evidence should be collected by as many biochemical methods as can be put into practice. Then judgment on the most probable sequence should be based on the cumulative evidence available. In the following subsections we shall consider some of the available methods for testing a hypothesis that a substance B is an intermediate in the conversion of an initial metabolite A into a product C under the agency of protoplasmic or enzymic systems e_a and e_b.

$$A \xrightarrow{e_a} B \xrightarrow{e_b} C$$

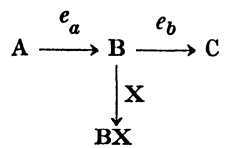
Qualitative analysis. Evidently B should accumulate if it is formed from A more rapidly than it is converted into C. Experiments should therefore be performed to discover whether B can be detected in cells which are converting A into C. The finding of B would be consistent with the hypothesis that it is an intermediate metabolite, but it would not afford definite proof, for it might originate during the course of some other reaction. The support

given would be stronger were it shown that B is present in cells only under conditions which permit the conversion of A into C.

Four examples must suffice. (*a*) The detection of fatty acids in cells in which fats are being converted into carbohydrates, or *vice versâ*, is consistent with the hypothesis that the acids are intermediate metabolites in these conversions. (*b*) The simultaneous occurrence in plants (see chap. XIV) of certain C_4 and C_6 acids suggests that these acids are readily interconvertible. (*c*) The fact that acetaldehyde has been detected among the products of fermentation, and of the zymasis component of the anaerobic respiration of higher plants, suggests that it might be the precursor of ethyl alcohol. (*d*) The detection by A. L. James (p. 208) of maltose in the embryo of germinating barley provides evidence that it is an intermediate product in the hydrolysis of starch to glucose in the endosperm, and that it migrates to the embryo.

Failure to detect B does not invalidate the hypothesis that it is an intermediate between A and C. Evidently it would only have a transient existence were it converted into C more rapidly than it is formed from A.[1] For example, (*a*) the formaldehyde hypothesis of photosynthesis must not be rejected simply because this aldehyde, although searched for intensively, has never been detected in illuminated green leaves; (*b*) we may still acquiesce in the view that acetaldehyde, pyruvic acid, or other products of zymase cleavage, are intermediate metabolites in the oxidative metabolism of carbohydrates, although these products are normally absent from plant cells; (*c*) the fact that maltose is absent from green amyliferous leaves does not exclude the possibility that it is formed during the hydrolysis of starch in the chloroplasts. If B does not normally accumulate use should, whenever possible, be made of the methods of fixation, differential inhibition of enzymes, and the separation of enzymes, in order to determine whether B has a transient existence.

Fixation methods. If a substance X, which can enter into chemical combination with B, is added to cells which are converting A into C, it will compete for B with the enzyme e_b, and some B may in consequence become fixed as BX.



The detection of BX under these conditions, although supplying important evidence that B is an intermediate between A and C, would not afford rigid proof of this hypothesis, seeing that B might

[1] If the substance (*e.g.*, foramldehyde, acetaldehyde) is a poison this rapid removal is an advantage to plant cells. Under certain conditions acetaldehyde accumulates in and poisons the flesh tissue of the apple, pear, medlar, etc.

be the product of some other metabolic change. Again, the evidence would be stronger were B to be fixed only when A is being converted into C. Even so, B might be derived from a substance D as a result of changed conditions brought about by the presence of X.

Neuberg has successfully applied this method to test the hypothesis that pyruvic acid and acetaldehyde have a transient existence during the alcoholic fermentation of sugar by yeast. Calcium pyruvate accumulated when the fermentation was carried out in the presence of calcium carbonate. Pyruvic acid was also fixed by von Graf (see p. 59). When sodium sulphite or dimedon [1] was added to the fermenting mixture, acetaldehyde was fixed (see Harden, *117*). Acetaldehyde bisulphite was formed with the first-named fixing agent, and acetaldomedon with the other. Ethyl alcohol also accumulated. Neuberg concluded that some of the acetaldehyde escaped fixation, and was reduced by enzyme systems in the cell.

James *et al.* (p. 303) employed hydrogen cyanide and sodium bisulphite as fixation agents in detecting the production of triosephosphates during glycolysis by barley saps. Neuberg and Gottschalk found that acetaldehyde bisulphite accumulated when sodium sulphite was added to ground pea meal under anaerobic conditions, and concluded that acetaldehyde was an intermediate metabolite in the anaerobic respiration of peas. Klein and Pirschle separated acetaldomedon from plant tissues that had been metabolizing in the presence of dimedon under aerobic conditions, and inferred that acetaldehyde is an intermediate product of oxidative metabolism, and possibly an intermediate product in the respiratory oxidation of carbohydrates (see p. 305).

Klein and Werner attempted to prove the formaldehyde hypothesis of photosynthesis by fixing formaldehyde as its aldomedon. They stated that they obtained formaldomedon when but only when photosynthesis was in progress. Doubt was thrown on their conclusions when Barton-Wright and Pratt (*18*) produced formaldomedon by an ordinary photochemical reaction in the absence of living cells, and simply as a result of illuminating carbonic acid in the presence of dimedon. Klein and Werner found that formaldomedon was never present inside living cells, and that it could not be detected in the external solutions when water plants were assimilating at low light intensities. Foster (*89*) confirmed and stressed the importance of these two findings, which are difficult to reconcile with the view that formaldehyde arises, in such experiments as these, from the photoreduction of carbon dioxide in the chloroplasts. Evidently much care is necessary in interpreting the results of some fixation experiments.

The differential inhibition or activation of enzymes in living cells. It is well known that the activity of enzymes may be influenced by altering the temperature, *pH*, or certain other factors, or by adding substances to the reaction medium. Evidently differential inhibition or activation of enzymes in living cells may lead to the accumulation of substances, which normally have only a transient existence. For instance, if we succeed in effecting the differential inhibition of e_b or the differential activation of e_a in the conversion of A into C, B would

[1] This word is a contraction for dimethyl-*cyclo*-hexanedione. In solution in the presence of aldehydes it gives rise to crystallizable addition compounds termed aldomedons. As solids, they may be distinguished by their melting points. Acetaldomedon melts at 140° C., and formaldomedon at 180° C.

tend to accumulate. It must be remembered, however, that abnorma chemical changes may occur in the presence of the inhibitor.

This virtually is the method employed when hypotheses are tested by autolysis experiments (p. 69). The rates of linked enzyme actions are differentially altered during the course of the disorganization of cells, and substances not normally present in notable concentrations may accumulate For instance, the fact that maltose may attain abnormally high concentrations when amyliferous leaves or germinating seeds are slowly dried at moderate temperatures is consistent with the view that it is a normal but transient intermediate in the hydrolysis of starch (*cf.* pp. 25 and 224). Maltase may be more active than diastase in the metabolism of healthy cells, and the reverse may hold during autolysis.

The accumulation of ethyl alcohol and acetaldehyde during the course of the autolysis of the senescent flesh tissue of apples stored in air (Fidler, *86*), or earlier in injured fruit (Thomas, *305*), has been ascribed to the differential inhibition of enzymes. It has been suggested that the activity of the oxidation enzymes was more depressed than that of zymase (*cf.* p. 213). These results are consistent with the view that the intermediate products of zymase cleavage are oxidatively consumed in healthy cells (see Blackman's schema, p. 308).

Chloroform, sodium fluoride, and sodium iodoacetate, are among the substances which, owing to their power of differentially inhibiting enzymes in the zymase complex, have helped to elucidate the chemistry of fermentation (pp. 54–61). The study of differential inhibitions, brought about by phenyl urethane, cyanides, sulphides, and carbon monoxide, have played an important part in the development of our knowledge of oxidative metabolism (pp. 31–7 and 312), and of glycolysis in the higher plants. Further, James *et al.* (p. 306) have effectively used 1-naphthol-2-sulphonic acid to inhibit carboxylase, and so to demonstrate the production of pyruvic acid by barley tissues and saps.

The demonstration of the presence of specific enzymes in living cells. The demonstration of the presence in living cells of specific enzymes e_a and e_b that can *in vitro* respectively convert A into B, and B into C, would afford strong support for the hypothesis that B is an intermediate metabolite in the conversion of A into C. There is no necessity to give illustrative examples here, for we have earlier (chap. III, section C) described the activities of some of the more important of the enzymes that have been separated from living cells. We also indicated possible sequences in zymase cleavage, in the hydrolysis of proteins, starch, amygdalin, and other condensates, and in certain syntheses, which have been suggested as a result of the resolution of zymase, proteases, diastase, emulsin, and other enzymes, into components showing specificity. As an illustrative example here, we note that the detection in a plant cell of phosphoglucomutase (p. 61) as well as phosphorylase (p. 62) would provide evidence that in that cell glucose-1-phosphate is an intermediate in the production of starch from glucose.

Feeding experiments. The demonstration of the simultaneous disappearance of B and appearance of C when B is fed to a tissue

which can convert A to C, would provide strong support to a hypothesis, advanced on the basis of other evidence, that B is an intermediate metabolite in this conversion. The evidence would be stronger were C produced from B as rapidly as from A. But such evidence would not be conclusive, because it is possible that B might give rise to D while at the same time causing a substance F to change into C. Obviously the single study either of the disappearance of B or of the appearance of C will not prove that these events are connected. It should be noted that if B does not disappear we cannot conclude that it is not produced in the conversion, since it might normally give rise to C by combining with another substance E, let us say, which, under natural conditions, is produced simultaneously and in commensurate amounts. Evidently unless E is also added in the feeding experiments, B would not disappear.

Feeding experiments of various types have been performed at intervals over many years, but with inconclusive results, to test the formaldehyde hypothesis of photosynthesis (see Stiles, *287*; Spoehr, *280*). Doubt has been thrown by Foster (*90*) on Bodnar's claim to have proved this hypothesis. Bodnar reported that living leaves or an enzyme in leaf powder could polymerize formaldehyde with the production of reducing sugars, but he did not measure formaldehyde consumption. Foster set out to show that such increases in reducing sugar as Bodnar had reported could be related to the amount of formaldehyde consumed, but found (*a*) that formaldehyde consumption was always very slight, and was a thermostable and not an enzyme phenomenon, and (*b*) that the amount of sugar produced was often greater in the control experiments, *i.e.*, in the absence of formaldehyde.

Unsuccessful attempts have been made to prove by feeding experiments that products of zymase cleavage undergo oxidative metabolism during the process of aerobic respiration (p. 304). But such experiments have proved informative in studying alcoholic fermentation. Most of the substances, placed in fig. 2 as intermediate between carbohydrate and ethyl alcohol and carbon dioxide, have been shown to be fermentable when fed to yeast.

Feeding experiments have been much used in the investigation of the metabolism of mould fungi and bacteria. For example, Allsopp's experiment (p. 337) suggests that gluconic acid may be an intermediate in the conversion by *Aspergillus niger* of glucose into oxalic acid, and Wood and Werkman's (p. 45) that pyruvic acid is a precursor of C_4 acids. Examples of the use of feeding methods in the study of nitrogen metabolism are given on pp. 199 and 204.

Suggestive evidence concerning the metabolism of illuminated and of darkened leaves has been obtained by feeding them with solutions containing various substances. We have already considered such evidence in discussing the synthesis of nitrogenous organic compounds (p. 199). It has long been known that certain leaves, which have previously been freed from starch, can manufacture this polysaccharide in the dark when they are floated on solutions containing one of the following substances: glucose, fructose, mannose, galactose, glycerol. The evidence does not appear to be conclusive that the starch is actually produced from the nutrient absorbed. The list given is not exhaustive. For example it has often been stated that feeding with cane sugar gives the best results, but

it is not known whether this substance undergoes hydrolysis prior to the formation ot starch. Since the hydrolysis of starch in wilting leaves, and in certain other organs (*e.g.*, potato tubers) when they are drying up, may result in the accumulation of cane sugar, we cannot exclude the possibility that starch may be converted into cane sugar without passing through an intermediate monosaccharide stage. This suggests that the reverse change may also occur, viz., the direct conversion of cane sugar to starch. If this happens, since starch is a glucosan and cane sugar contains fructose, we could infer that fructose units are converted into glucose units, unless a fructosan is produced simultaneously with starch. Feeding experiments quantitatively carried out may help to clear up the confusion in our present knowledge of this important question of the relation between starch and cane sugar. We note that the evidence cited above suggests that green leaves can effect, in the dark, intramolecular conversions within the carbohydrate group, since starch was produced by leaves floated on fructose, mannose, or glucose. Virtanen and Nordlund (*318*) have more recently reported that cane sugar was synthesized in the dark by leaves of red clover and of wheat plants when they were floated on a ten per cent. solution of either glucose or of fructose.

The experimental results that we have just considered are consistent with the theory that soluble hexoses are the primary products of photosynthesis, and that the formation of cane sugar and starch occur as secondary processes independently of the presence of light (*cf.* p. 197). The necessary or critical concentration of sugar for starch formation varies in different plants. In certain dicotyledonous leaves (*e.g.*, those of plants belonging to the Solanaceae and Leguminosae) the minimum concentration for starch formation may be less than 0·5 gm. per 100 gm. of fresh weight leaf. In many monocotyledonous leaves concentrations greater than fifteen per cent. must be present before starch formation can be induced. Since these leaves do not normally form starch when they are illuminated, further evidence is afforded that starch formation is only dependent on photosynthesis in so far as this process provides sugars for condensation with dehydration, or glucose phosphate for condensation with dephosphorylation (p. 63).

We note that the fact that plant cells can convert glycerol into carbohydrates accords well with the view that glycerol is an intermediate metabolite in the conversion of fats into carbohydrates. This feeding experiment has provided direct evidence that there is a metabolizing system in protoplasm that can effect this conversion.

To obtain satisfactory results in feeding experiments of the kind we have described, it is necessary to ensure that leaves receive an adequate supply of oxygen. This point has been emphasized by Phillis and Mason (*230*). These workers reported that some starch was formed even under anaerobic conditions in the dark when discs cut from leaves of the cotton plant were floated on cane sugar solutions, but that the rate of production of starch was greatly enhanced in the presence of oxygen. Moreover, they maintain that dull light, independently of any action it might have had on photosynthesis (they carried out their experiments in an atmosphere free from carbon dioxide), very strongly augmented the rate of starch formation. They concluded from the results of quantitative experiments that light and oxygen act by promoting the absorption of sugar by green cells.

The accumulation sequence. Views concerning the sequence of stages in metabolism may sometimes be tested by determining the order in which possible intermediates appear under experimental

conditions. For example, Weevers (see Stiles, *287*) has investigated the problem of the first sugar formed in photosynthesis by determining the production sequence of carbohydrates during this process. Leaves of *Pelargonium zonale* were placed in the dark until they were free from starch, cane sugar and hexose sugars. The leaves were then illuminated, and from time to time analysed. Carbohydrates appeared in the following order: hexoses, cane sugar, starch. Weevers concluded that hexoses are the precursors of cane sugar. We cannot decide from this experiment whether the starch is derived from the hexoses or from the cane sugar. Chemical considerations would favour the view that it is formed from glucose or glucose phosphate, but certain other recent experiments suggest that starch and cane sugar may be readily interconvertible (see Onslow, *214*, chap. I).

Privative methods. Important evidence concerning possible metabolic sequences has been obtained by excluding from the reacting system one factor that is normally operative. Well-known examples of the application of this method are the comparison of aerobic and anaerobic events, the investigation of metabolism in illuminated and darkened leaves, and the study of the chemical behaviour of plants growing in culture solutions deficient in one of the essential elements.

CHAPTER XIII

CARBON ASSIMILATION OR THE PHOTOSYNTHESIS OF CARBOHYDRATES

A. Experimental Methods

General considerations. Carbon assimilation or photosynthesis consists in the production by illuminated chloroplasts of hexose sugars from carbon dioxide and water. Oxygen is formed as a by-product.

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

The carbon dioxide passes into the green leaf through the stomata (chap. X), and the water obtained from the soil reaches the leaf by way of the conducting parenchyma of root and leaf, and the vessels and tracheides of the xylem (chap. VIII). Oxygen passes out through the stomata. A fraction of the hexoses formed may accumulate; the remainder is variously metabolized without the intervention of further light energy. One part undergoes condensation to yield higher carbohydrates (*e.g.*, cane sugar, starch); another part is used in the production of aromatic compounds, proteins, and other compounds; and all the while some of the carbohydrates, and possibly other compounds, undergo respiratory oxidation. Furthermore, material is lost to green leaves by the translocation in the phloem of certain crystalloidal metabolites such as cane sugar, and "residual nitrogen," to other metabolizing tissues in the shoot or root.

By synthesizing organic compounds, leaves increase their carbon content, dry weight, and energy content. These increases are opposed by respiration in detached leaves under experimental conditions, and by translocation as well as by respiration in attached leaves.

The methods used in studying photosynthesis, some of which are outlined below, consist essentially in the measurement either of the gaseous exchange or of the accumulation of organic compounds associated with the process, due allowance always being made for respiration and translocation.

The measurement of CO_2 uptake. In order to determine the average amount of carbon dioxide absorbed from ordinary air in one hour of a summer's day by a given sample of leaves, equal volumes of air are simultaneously passed for a period (*a*) over the green leaves exposed to light and then through a solution of baryta, and (*b*) directly into an equal volume of the same solution of baryta as that used in (*a*). If the soda-lime tube for freeing the air from carbon dioxide is omitted, the apparatus for measuring respiration, described on p. 262, and illustrated in fig. 33, may be used. At the end of a given period, both of the baryta solutions should be titrated against standard hydrochloric acid. The average apparent assimilation per hour

(*i.e.*, the CO_2 uptake) by the sample under the conditions of the experiment may be calculated from the difference between the two titrations. To evaluate the real assimilation, a correction for the average hourly respiration of the same leaves at the same temperature must be applied. The chamber containing the leaves should be placed in the dark and the respiration of the leaves measured (p. 261). The real assimilation is then obtained by adding together the value of the CO_2 uptake (*i.e.*, the apparent assimilation), and the amount of carbon dioxide produced in respiration.

The results may be expressed in a variety of ways. The carbon dioxide absorbed in unit time (usually one hour) may be given in cubic centimetres or in grammes ; the temperature is always recorded in degrees centigrade ; the light intensity is sometimes given absolutely as metre candles (lux units), but statements of relative intensities are often sufficient. The percentage of carbon dioxide in the gas mixture surrounding the plant during the course of the experiment should also be noted. According to the purpose in view, photosynthesis is referred to the area of leaf surface, or to the fresh weight or the dry weight of leaves. The experiments are beset with difficulties, as all these magnitudes may change during the course of an experiment. Critical discussion would, however, take us too far.

The measurement of CO_2 absorption has been the method most frequently used in quantitative researches on photosynthesis. Water plants provide more suitable experimental material than green shoots or leaves of land plants, since in the latter the rate of supply of carbon dioxide to the chloroplasts from a gas mixture of constant composition may alter during the course of the experiment owing to stomatal movements (p. 159). Special pieces of apparatus have been designed by F. F. Blackman, Spoehr, and others, for measuring photosynthesis by water plants in moving water containing known percentages of dissolved carbon dioxide or of sodium bicarbonate (see Stiles, *287*, and Spoehr, *280*). Sodium bicarbonate is either itself assimilated or becomes dissociated into carbon dioxide, which is then assimilated.

The measurement of oxygen output. The output of oxygen by green leaves in a closed glass vessel exposed to light may be determined by gas analysis with Haldane's apparatus (p. 264), and the output by small objects, such as Chlorella cells, with the aid of some modification of the Barcroft-Warburg apparatus (p. 263). Oxygen output, however, is usually demonstrated by collecting and testing the gas which bubbles from cut surfaces of illuminated green shoots of water plants (*e.g.*, Canadian waterweed (*Elodea canadensis*)). The gas given off is not pure oxygen, but is much richer than air in oxygen. The reason for the bubbling during photosynthesis is that oxygen is much less soluble than carbon dioxide in water. The oxygen goes out of solution and into the intercellular spaces as a gas, and increases the gas pressure in these spaces. Consequently the air, enriched in oxygen, escapes in the form of bubbles from cut surfaces.

The rate of bubbling may be used as a measure of the relative rate of photosynthesis under different external conditions. The CO_2 supply is conveniently varied by using solutions of sodium bicarbonate of different strengths. This compound by ionic dissociation finally provides carbon dioxide for photosynthesis. Light intensity and temperature may also be altered. The same piece of shoot must be used for a set of comparative experiments, and arrangements must be made for the discharge of bubbles of a uniform size. For research purposes special bubblers have been designed, but for class experiments it is sufficient to cover the cut surface of the shoot with gelatine, and then to pierce the covering with a needle.

Care must also be taken that the shoot always presents the same surface towards the light source.

In certain important researches the oxygen output of illuminated leaves has been measured by F. F. Blackman's palladium method. The leaves were initially surrounded with a gas mixture of hydrogen and carbon dioxide. After a period of illumination the surrounding gas, which would then also contain oxygen, was passed over a palladium surface, on which all the oxygen and some of the hydrogen combined to give water. From the resulting reduction in volume, which was measured eudiometrically, the oxygen output was calculated.

Of the other methods which have been used for detecting the evolution of oxygen, two delicate bacterial methods may be mentioned. In one, the fact that luminous bacteria are luminous only in the presence of oxygen has been exploited. In the other, which has been more frequently used, motile bacteria, such as *Bacillus termo*, are enclosed with green tissue in water under a sealed coverslip. In the absence of oxygen these bacteria do not move, but begin to do so in the presence of a mere trace of oxygen. Hill (*129*) has used hæmoglobin in an evacuated Thunberg tube to measure spectrographically the appearance of small quantities of oxygen when cell-free suspensions of chloroplasts were illuminated in the presence either of leaf extracts or of certain ferric salts (see pp. 12 and 236).

The measurement of dry weight increase. It follows from the equation given at the beginning of this section that the dry weight of plants increases by 180 gms, when hexoses are photosynthesized from 264 gms of carbon dioxide and 108 gms of water. It has been calculated that over ninety per cent. of the dry weight of plants is due to photosynthesis. Sachs's half-leaf method of measuring photosynthesis is based on this fact. Although this method is not now used in exact experiments it instructively illustrates important functions of green leaves, and thus merits mention.

At the beginning of a period of illumination, the dry weight is determined of a definite area cut out from the half blade on one side of the midrib of each leaf in a sample. Each leaf selected should have similar venation on the two sides of the midrib. Then at the end of the period the dry weight is determined of an equal area of the same shape removed from a similar position on the other half-blade of the leaf. Let w_1 represent the average dry weight of the areas removed at the beginning and w_2 that of the areas removed at the end of the experiment. Then $(w_2 - w_1)$ measures the amount of substance that has accumulated in these areas during the period But substances have throughout been removed from these areas by respiration in detached leaves, and by both respiration and translocation in leaves normally attached to the stem. The average amount removed is measured by using the half-leaf method at the same temperature with another sample of leaves, and determining the average loss in dry weight $(w_3 - w_4)$ during an equal period in the dark. Clearly, on the average, the amount of dry matter formed by photosynthesis in the area used during the period of illumination would be $(w_2 - w_1) + (w_3 - w_4)$. This is usually expressed in grammes increase of dry weight in unit time per unit area of leaf surface.

To obtain reliable results large samples must be used ; even so, there are many sources of error. For example, it has been shown that, owing to changes in their water content, leaves may significantly alter in area during periods of illumination and darkening. Hence the number of photosynthesizing cells in a given area of leaf may fluctuate during the course of a day. This introduces grave errors, seeing that what we really require to know in our quantitative experiments is the change in the dry weight of a

given number of comparable mesophyll cells over a definite period in the light and in the dark. For a given area it has been shown that even after allowing for respiration and translocation, the dry weight increase, as found by the half-leaf method, is rarely what would be expected from the amount of carbon dioxide absorbed during the same period.

The measurement of the increase in energy content. The increase in the dry matter and in the energy content of green leaves during photosynthesis have been simultaneously determined by the half-leaf method. It appears that for every gram of substance accumulating in leaves during photosynthesis, 4–5 Kilogram-Calories of energy are fixed. This figure represents w_1 and w_2 (see the last sub-section), which are obtained in the dry weight determinations.

TABLE XI. *Heats of combustion of* 1 *gm. of various metabolites in Kg.-Cals.*

Glucose	3·79	Leucine (an amino-acid)	6·5
Cane sugar	3·99	Vitellin (a protein)	5·7
Starch	4·1	Linseed oil	9·47
Cellulose	4·2	Simple benzene compounds	10

It is clear from the data given in table X, that values for energy fixation greater than 4·2 Kg.-Cals. per gm. dry weight indicate that substances other than carbohydrates, and possessing a higher calorific value per gm. dry weight, must accumulate in leaves during photosynthesis. It should be noted that none of these substances is a direct product of photosynthesis. Nevertheless each secondary product derives all its energy from the solar radiation absorbed in photosynthesis. The solar energy fixed, however, is entirely transformed into the potential energy bound within the molecules of the hexoses that are produced. These hexoses are then variously changed; a fraction is condensed to higher carbohydrates, a change involving a slight increase in energy content per gram (see table X); and other fractions take part in the production of proteins, lipoids, benzene compounds, and other substances possessing a higher energy content per gram than that of the hexose from which they are derived. It is supposed that this extra energy comes from the chemical energy set free by the respiratory oxidation of yet another fraction of the hexoses formed by photosynthesis. If it does, all the chemical energy bound in green leaves is derived either directly or indirectly from the sun's rays. It should be noted that, owing to the continuous respiratory oxidation of organic compounds during a period of illumination, the total amount of energy in a leaf at the end of such a period is less than the amount of energy that is actually fixed by the photosynthesis of hexose during that period, *i.e.*, the energy fixation equivalent of apparent assimilation is less than that of real assimilation.

Experiments have shown that less than one per cent. of the total light energy incident upon green leaves may be used in photosynthesis. The remainder is reflected from the leaf surface or transmitted through the leaf or changed into heat. The temperature of an insolated leaf may become higher than that of the air, but sooner or later the extra heat is either absorbed in transpiration as the latent heat of evaporation of water, or re-radiated into the air. Attempts have been and are still being made to judge the efficiency of chloroplasts in using the small fraction of the incident light energy that they absorb. The photosynthetic efficiency of a tissue is defined as the ratio of the amount of energy fixed per unit time, *i.e.*, changed into the chemical energy of the organic products of photosynthesis, to the light energy absorbed in unit time by the chloroplasts. Experimental

results suggest that although very little of the incident light is absorbed by the chloroplasts, fifty per cent. or more of that which is absorbed may, under favourable conditions, be used in photosynthesis, *i.e.*, the efficiency may be greater than 0·5.

The measurement of increases in the amounts of carbohydrates.[1] Theoretically, photosynthesis might be measured by determining the rate of production of any organic substance that accumulates at a rate proportional to that of CO_2 uptake; but, actually, little attention has been given to substances other than carbohydrates.

It is not difficult to show (*a*) that glucose, fructose and cane sugar are generally present in green leaves, and that the total concentration of sugars increases during a period of illumination; and (*b*) that starch simultaneously accumulates in the assimilatory cells of many plants. In some species, however, particularly among the Liliaceae and Amaryllidaceae, starch is never found in green cells.

(*a*) *The estimation of sugars.* Sugars may be extracted from fresh leaves by plunging the leaves into boiling alcohol. This supplants an older method in which leaves were dried in warm air before extracting the sugars. It was found that owing to the persisting activity of enzymes autolysis takes place during drying, and changes occur in the relative amounts of various carbohydrates. Thus maltose, which is not normally present in leaves, is formed in drying leaves by the action of diastase on starch (p. 216). Plunging into boiling alcohol immediately inactivates enzymes, and consequently the amounts of carbohydrates finally found are the same as those which exist in the fresh leaves.

The alcoholic solution is evaporated to dryness, and the residue taken up in tepid water. Next, the glycosides, amino acids, and certain aromatic substances, are precipitated by adding basic lead acetate to the aqueous extract. A solution of sugars, fairly free from other organic impurities, is obtained by filtration. Any excess of the lead salts is removed by precipitation, and the total amount of reducing sugars in the filtrate is ascertained by titrating part of it with Fehling's solution, or by some other volumetric method. The glucose in another part is oxidized with an alkaline solution of iodine, and the residual reducing sugar (viz., fructose) is estimated as before. Total reducing sugar less fructose gives glucose. To another part sufficient citric acid crystals are added to give a ten per cent. solution, and the mixture is boiled for ten minutes. The cane sugar is thus completely hydrolyzed to glucose and fructose. This solution, which will contain the original reducing sugars and those formed by the hydrolysis of cane sugar, is neutralized with sodium carbonate. The total amount of reducing sugars present in this solution is then determined volumetrically. The amount of cane sugar present in the original extract from leaves may be calculated from the increase in reducing power brought about by hydrolysis with citric acid.

(*b*) *The estimation of starch.* During the extraction of sugars, any starch that has been produced in photosynthesis remains in that fraction of the leaf residue which is insoluble in alcohol. Theoretically, the starch present in this sugar-free residue may be estimated by determining the amount of reducing sugars formed upon hydrolysis. Takadiastase, a special form of diastase prepared from the mycelium of *Aspergillus oryzae*, has been used in several researches. This enzyme converts starch into a mixture of maltose and glucose. Acid hydrolysis has also been used. With this method, however, error may arise through the cleavage of other poly-

[1] For details concerning older methods of estimating carbohydrates see Haas and Hill (*108*), and concerning new methods see Barnell (*15*), Yemm (*337*).

saccharides, such as hemicelluloses, fructosans, and pentosans, and corrections must be made.

Starch is the most easily detected of all the substances which may accumulate during photosynthesis. Thus individual starch grains, stained deep blue, are seen in the chloroplasts of sections of green starch-forming leaves mounted in chloral-hydrate-iodine (Schimper's solution). The presence of starch in whole leaves is detected by the blue colour given when leaves, which have been decolourized by placing them first in boiling water and then in ethyl alcohol, are treated with a solution of iodine in potassium iodide.

For class work, these methods being simple, may be used to ascertain the necessary conditions for photosynthesis, and as a measure of the rate of this process. For example, let us suppose that clover plants, the leaves of which have previously been shown to contain starch, are placed in the dark until most of the leaves have become starch-free as a result of hydrolysis and of other processes which are discussed elsewhere (p. 199). The plants may then be placed in the light in the absence of carbon dioxide, or in various conjunctions of air and carbon dioxide, or in ordinary air under the same illumination but at different temperatures, or in ordinary air at the same temperatures but under different illuminations. After equal periods under different experimental conditions, large samples of leaves are taken from each plant. The leaves are first decolourized, and then placed in iodine. The relative amount of starch which has been formed in each leaf is then judged by the intensity of blue given. For example, leaves may be classified as giving no reaction, medium reaction, strong reaction, and very strong reaction. For any one set of conditions it will be found that variation usually occurs for a single plant. The most frequently occurring reaction should be taken as representing the amount of starch formed in that plant during the experiment.

B. The Photosynthetic Reacting System

The necessary factors. It can readily be shown by the methods described in the last section what external factors must be operative before photosynthesis will take place in a given green plant. Thus for starch formation, or for the production of bubbles by submerged shoots, there must be a supply of carbon dioxide, a suitable temperature, and light of the requisite wave length. Photosynthesis ceases at temperatures considerably less than 50° C. The deceleration and stoppage of physiological processes at temperatures greater than the so-called optimum are discussed on p. 282. The remarks made there are, in general, also applicable to photosynthesis.

Towards the end of the eighteenth century it was established that only green organs can absorb carbon dioxide and give off oxygen. Much later it was shown that non-green tissues, even when they are illuminated, cannot produce carbohydrates from carbon dioxide and water. Thus starch is produced from carbon dioxide and water only in the green cells of illuminated variegated leaves. There is strong evidence that this power of photosynthesis, which is uniquely possessed by green cells, resides in the chloroplasts, and only in these structural units of green living cells. Thus the starch that accumu-

lates in green cells during photosynthesis is always confined to the chloroplasts. And it has been shown by the motile bacteria method that only the spiral chloroplasts of illuminated filaments of Spirogyra give off oxygen. Several investigators have reported that phenomena attributable to photosynthesis occurred when non-nucleated fragments of protoplasm containing chloroplasts were floated in a solution of carbon dioxide and exposed to light, but it has not yet been established that chloroplasts can act alone. Modern methods of microdissection have not yet been applied to reinvestigate the important problem of what is the least structural unit in green cells which can effect photosynthesis. Chemical and physiological methods have, however, advanced our knowledge of the internal factors that are operative in whatever this structural unit may be.

The chloroplast pigments. According to Willstätter and Stoll, four pigments are universally present in chloroplasts; two of these, chlorophyll *a* and chlorophyll *b*, are green, and of the non-green pigments, carotin is orange coloured and xanthophyll is yellow.[1] The relative amounts of these pigments in normal green leaves of different species of land plants do not vary greatly. Weight for weight, the green pigments are about ten times as abundant as the yellow, and in molar proportions the ratio amount of chlorophyll *a*/ amount of chlorophyll *b* is 3/1, and the ratio amount of xanthophyll/ amount of carotin is 1·5/1. In gold-leaved varieties of plants, however, such as those of the elm, elder, and oak, the concentration of the yellow pigments exceeds that of the green.

Both before and for some years after the experiments of Willstätter and Stoll were performed, it was generally believed that the pigments extracted from green leaves by acetone or alcohol, and dissolved in these or other lipoid solvents, or colloidally dispersed in water, are chemically identical with the pigments as they exist in living chloroplasts. But it was known that there are always important differences between the properties shown by chlorophyll extracted from a green leaf and those displayed by the green pigment in living chloroplasts. For example, the green pigment is photostable when in the chloroplast, and shows only a weak fluorescence; whereas chlorophyll dissolved in a lipoid solvent produces a strong red fluorescence, and readily undergoes photodecomposition. Moreover, there are marked differences between the positions of the maximum absorption bands seen when the light passing through green leaves on the one hand, and through solutions in lipoid solvents of extracted pigments on the other, is examined with a spectroscope. The solutions in lipoid solvents show absorption bands nearer the blue-violet end of the spectrum. Consideration of these points of difference have shown to be untenable several hypotheses which have been put forward concerning the physical state in which pigments, assumed to be chemically identical with extracted pigments, might exist in living chloroplasts (see Stiles, *287*; Hubert, *143*). A straightforward idea advocated for many years was that in living leaves chlorophyll is dissolved in the lipoid substances that occur in chloroplasts.

[1] For accounts of the chemistry of these plastid pigments, see Appendix I.

But this idea has been discredited because the fluorescence of leaves, unlike that of lipoid solutions of chlorophyll, is very weak; indeed, so weak that it escaped detection for many years. An interesting point to note is that the fluorescence may be augmented by plunging leaves into boiling water; possibly in killed leaves chlorophyll does actually dissolve in the lipoids of the chloroplasts. Moreover, the absorption bands shift towards the blue-violet end of the spectrum. Willstätter and Stoll suggested that chlorophyll is dispersed as a hydrosol colloid in the protoplasmic basis of chloroplasts. In favour of this view is the fact that the maximum absorption band in the spectrum of a colloidal solution of chlorophyll, especially in the presence of bivalent ions, is not far removed from that of living leaves. This band is situated near the wave-length 6,810 Ångstrom units, *i.e.*, 681 $\mu\mu$. But such a colloidal solution does not show even weak fluorescence.

The results of more recent work indicate that the problems must be stated in new terms, since evidence exists that during the extraction of pigments with acetone or other lipoid solvents a complex coloured substance is decomposed, setting free the pigments to which chemical formulæ were given by Willstätter and Stoll. For instance Lubimenko reported that he prepared from autolyzing aspidistra leaves a colloidal solution of a green chromoprotein, which possessed the same absorption spectrum as the green leaves themselves. This coloured substance could be flocculated with ammonium sulphate and still retain its identity, and could be cleaved in a number of ways into a colourless protein and pigmented substances. Lubimenko, therefore, concluded that true chlorophyll is a conjugate protein. He attached importance to certain specific variations (*e.g.*, of absorption spectra) that he observed in leaves, and attributed these to variation in the protein component of the chromoprotein, true chlorophyll.

Hubert (*143*), however, refers the variation reported by Lubimenko to errors resulting from the use of a microspectroscope that had an eye-piece with small dispersion. Hubert maintains that the maximum absorption band for living green cells, whether of algæ, vascular cryptograms, or flowering plants, occurs at about 6,810 Ångstrom units. In Noack's school at Berlin and Baas Becking's school at Leiden, evidence has, however, been obtained which supports Lubimenko's conclusion that chlorophylls *a* and *b* exist in a living leaf as prosthetic groups of a chromoprotein. By grinding leaves with water and then centrifuging, Noack obtained a green sediment, which showed a slight fluorescence resembling that displayed by living leaves. This fluorescence was lost by heating for thirty seconds at 75° C., the minimum temperature for the denaturation by heat of proteins. A fluorescent *fresh sap* containing fragments of chloroplasts was also prepared. This sap lost its fluorescence when treated with an active proteolytic enzyme. The fluorescence persisted in the green proteinaceous sediment thrown down when the sap was flocculated with ammonium sulphate or with lead acetate. The inference drawn was that the chromoprotein had been precipitated without denaturation. When, however, this precipitate was heated the fluorescence disappeared. The protein had been denatured. Fluorescence appearing subsequently was attributed to solution in lipoids adhering to the sediment.

The colouring matter of chloroplasts, whether this be a chromoprotein or a mixture of green and yellow pigments, is associated in chloroplasts with a colourless cytoplasmic stroma, the composition of which we have already discussed (p. 12). Hubert stresses the importance of the part played by lipoids as well as by protein and pigments in determining the make-up of chloroplasts. His picture is that in the plastids chlorophylls

a and *b* are chemically combined with protein, and that forces of cohesion hold in close association these chromoprotein molecules, lipoid molecules, and molecules of the carotinoids (fig. 27). The name phyllochlorin has been given to a single complex containing all these chemical units. A regular pattern results from a definite orientation of molecules according to their polarity, the hydrophilic porphyrin ends of monomolecular layers of chlorophyll being directed towards protein, and the hydrophobic ends

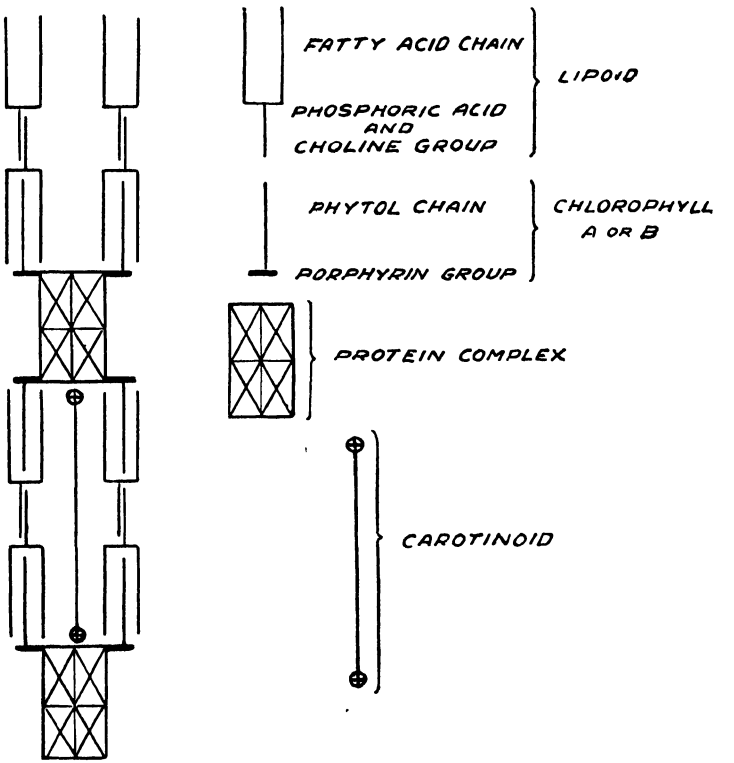


FIG. 27. Diagrammatic representation of Hubert's ideas, which are discussed in the text, concerning the possible disposition in the chloroplasts of molecules of chlorophyll, protein, lipoid, and carotinoid. There may be many other compounds in chloroplasts (see p. 12).

(which are also lipophilic) towards lipoid. There may be a considerable number of such layers within the cytoplasm of the stroma.

From microscopical observations, Zirkle concluded that chloroplasts are vacuolated porous structures enclosed in clear colourless cytoplasm, and that the colouring matter is *uniformly* distributed in colloidal dispersion in the cytoplasmic matrix of the stroma. Certain earlier observers maintained that the pigments are *confined to the surface* of the colourless stroma. Doutreligne observed in her microscopical preparations a large number of distinct pigmented granules, which were situated in the outer shell of the chloroplasts and embedded in the stroma. Von Euler's measurements

indicate that a single plastid of volume 40 μ^3 may contain more than a thousand million molecules of chlorophylls *a* and *b*. This means that there would be distributed in each pigmented granule of a chloroplast innumerable pigmented complexes of the type that Hubert pictured. The hypothesis that guided work in Baas Becking's school at Leiden in the 1930's appears to have been that each granule contains a number of pigmented

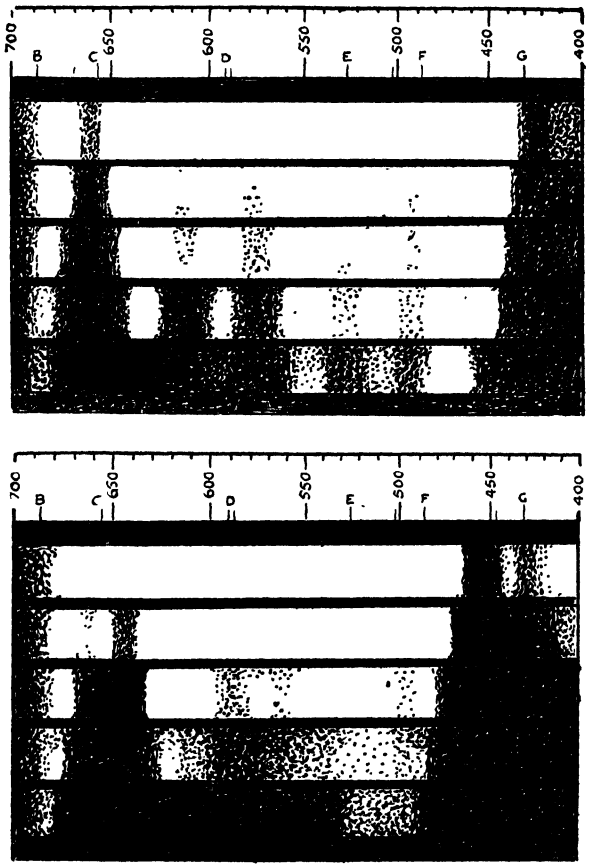


FIG. 28. The absorption spectra of five different concentrations of solutions in acetone of chlorophyll *a* (upper figure) and chlorophyll *b* (lower figure). (From Willstätter and Stoll.)

layers alternating with non-pigmented layers. Accordingly, for the further elucidation of the physical structure of the chloroplast, much attention was given to the study by modern methods of physical chemistry of the properties of chlorophyll multifilms (see *e.g.*, Nicolai and Weurman, *209*).

The most probable explanation of the fact that photosynthesis only occurs in green leaves is that the absorption of light energy for

the conversion of carbon dioxide and water into carbohydrates is dependent on the presence of the chloroplast pigments. Since the absorption spectra of green leaves and of chlorophyll *a* and chlorophyll *b* (fig. 28), although not identical, show the deepest bands in that range of wavelength of light (650–700 $\mu\mu$) in which photosynthesis proceeds fastest, we may infer that the green pigments are chiefly responsible for absorbing the light energy used in photosynthesis. It should be noted that light within this range is not absorbed by the non-green plastid pigments, carotin and xanthophyll (see fig. 29). This seems to exclude the possibility that the energy absorbed by these pigments is necessary for the occurrence of photosynthesis. Instructive experiments may be performed by placing green shoots

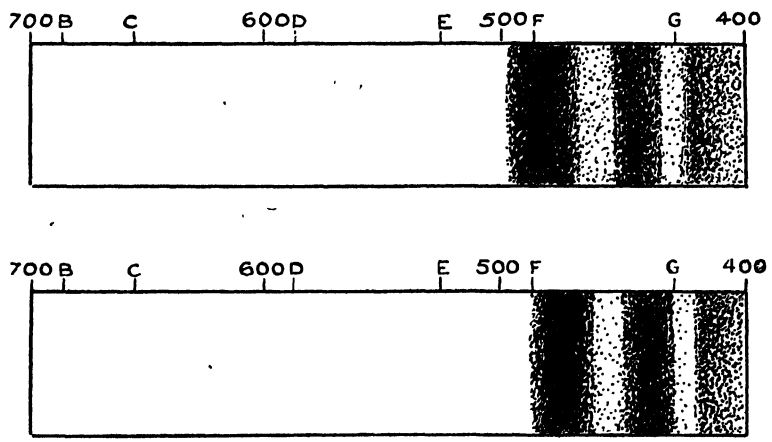


FIG. 29. The absorption spectra of solutions of carotin (upper figure) and xanthophyll (lower figure).

in double-walled bell jars containing coloured solutions, and judging the rate of photosynthesis by the starch formation, bubbling, or some other method. The results obtained by recent investigators appear to be in general agreement with Reinke's (fig. 30). In recent researches, colour filters such as are used in photography have proved of service in the determination of the photosynthetic efficiency of different wavelengths of light. Briggs (*51*), who employed Wratten gelatin filters in his experimental determinations by means of the palladium method of the rates of photosynthesis in yellow-red (570–640 $\mu\mu$), green (510–560 $\mu\mu$), and blue (430–510 $\mu\mu$) light, took the number of cubic centimetres of oxygen produced per 500 calories of incident light energy as a relative measure of the efficiencies of the different ranges of wavelength, and found that there is a " decrease of efficiency per incident energy in passing from the red to the blue end of the spectrum."

Protoplasmic or enzymic factors. It is very important to remember that photosynthesis is not wholly governed by the colloidally dispersed pigments. The cytoplasmic basis of the chloroplast plays some essential part in the process. No one has as yet succeeded in effecting photosynthesis *in vitro* under the agency of the free pigments separated from the chloroplasts. Experiments have been made with pigments dissolved in oil; Willstätter used pigments colloidally

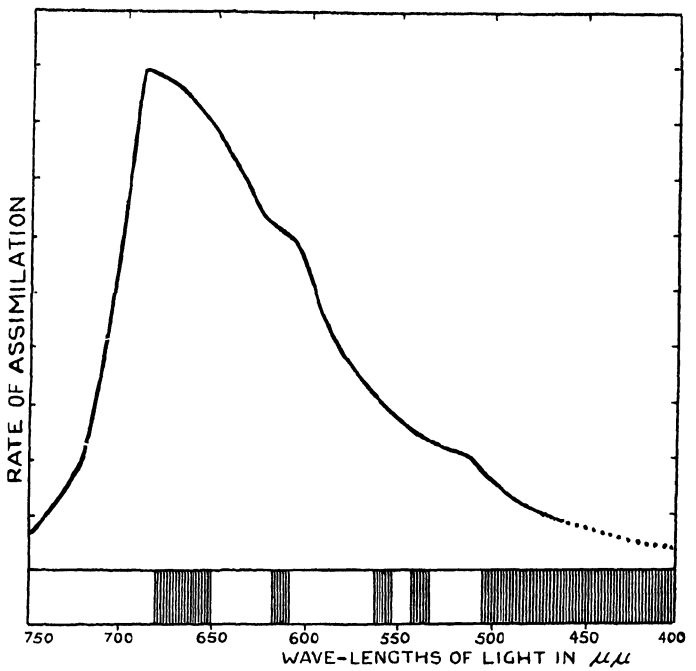


FIG. 30. The rate of assimilation, as determined by the rate of evolution of gas bubbles, compared with the absorption spectrum of a living leaf. (After Reinke.[1])

dispersed in water saturated with carbon dioxide; and Lubimenko made the attempt with the chromoprotein he separated from the leaves of aspidistra.

Further evidence that photosynthesis is not a simple photochemical reaction between chloroplast pigments, carbon dioxide, and water, has been gathered from experiments in which the process was retarded or even completely inhibited by modes of treatment that are supposed not to affect the pigments. Both Willstätter and Spoehr have reported that photosynthesis stops in leaves which have been

[1] See Jost (*158*), p. 128.

completely deprived of oxygen. Possibly the energy from aerobic respiration is essential in addition to light energy; but it should be noted that the presence of a trace of oxygen allows photosynthesis to proceed. There is evidence, however, that some non-pigmented protoplasmic system other than the respiratory system plays a part in photosynthesis. Thus it has been shown that photosynthesis ceases at temperatures between 40°–50° C., before respiration is completely inhibited, and that it is more readily narcotized than respiration by weak solutions of ether, phenyl urethane, potassium cyanide, etc. Since, after such forms of treatment, the chloroplast pigments remain unaltered, it follows that there is a thermolabile, narcotic sensitive component of the photosynthesizing system, which can be put out of action before respiration fails.

The investigations of G. E. Briggs (*49* and *50*), which extended the earlier work of Miss Irving, have provided the most convincing evidence for the existence of a non-pigmented internal factor. He found that the first assimilating leaves of certain seedlings, such as those of the runner bean (*Phaseolus vulgaris*, var. *multiflorus*), and of other plants in which the first assimilating leaves had not previously been storage cotyledons, turned green some time before photosynthesis began. He attributed this lag to the slower development of the necessary protoplasmic factor. He performed critical experiments on the first leaves of runner bean seedlings by the palladium method, and exploiting the well-established facts that chlorophyll does not develop under any conditions in the absence of oxygen or in the absence of light, succeeded in measuring photosynthetic activity from day to day in a pale green leaf in which the amount of chlorophyll, remained constant. He measured the rate of photosynthesis by determining the oxygen output of the leaves when illuminated by a light source of constant intensity, and placed in gas mixtures of hydrogen and carbon dioxide, *i.e.*, chlorophyll development was inhibited by the absence of appreciable quantities of oxygen. Between the experiments, the leaves were placed in air in the dark, *i.e.*, chlorophyll development was inhibited by the absence of light. Briggs found that for a given leaf under defined external conditions of temperature, light intensity, and CO_2 concentration, the photosynthetic power increased from day to day. Since the amount of chlorophyll remained the same, he inferred that this increase was due to the development of a necessary protoplasmic factor. He also measured respiration and concluded that the increase in oxygen output was considerably greater than could be accounted for by diminished respiratory activity in ageing leaves.

Willstätter and Stoll argued from the results of some of their experiments that the rate of photosynthesis is governed by an enzymic factor as well as by the amount of chlorophyll. Endeavour-

ing at the outset to relate the rate of photosynthesis under favourable external conditions to the concentration of chlorophyll, they obtained assimilation numbers (*i.e.*, the ratio, rate of CO_2-absorption/chlorophyll content in unit mass of leaf) (*a*) for different species of plants, (*b*) for golden leaved and green leaved varieties of the same species, and (*c*) for leaves of the same variety at different stages of development. The general conclusion drawn was that photosynthetic activity is usually not proportional to the concentration of chlorophyll, and it was inferred from this non-proportionality that photosynthesis is governed by an enzyme as well as by green pigment. Thus, for certain developing green leaves, which were exposed to high light intensities and received ample supplies of carbon dioxide, it was found that whereas the rate of photosynthesis increased with greening, the assimilation number (*i.e.*, the rate of photosynthesis per unit mass of chlorophyll) decreased. In some instances green leaves containing different amounts of chlorophyll photosynthesized at about the same rate. Green and golden varieties (*e.g.*, of elm) were also compared. The rate of photosynthesis of green leaves was usually but not always greater than that of the green-yellow leaves of the golden variety, but the assimilation numbers for the former were always much less than for the latter (see also p. 241). Stiles (*287*) and Spoehr (*280*) have summarized this work, and Briggs (*49*) has developed important arguments from some of the experimental results.

From the results of their experiments on photosynthesis by Chlorella in flashing light, Emerson and Arnold (*81*) inferred that for the reduction of one molecule of carbon dioxide a photosynthetic unit containing about 2,500 molecules of chlorophyll is operative. Since Willstätter and Stoll have shown that, at high light intensities, green-yellow leaves may display photosynthetic activity that is as great or even greater than that of fully green leaves, it is difficult at present to attach general significance to a quantitative definition of a photosynthetic unit in terms of the number of chlorophyll molecules. It is not denied that photosynthetic units may exist. Warburg. obtained experimental evidence that four quanta of light energy are used in the reduction of one molecule of carbon dioxide. More recent experiments suggest that eight quanta may be required. It may well be that each photosynthetic unit in a chloroplast is responsible for the effective absorption of these quanta. But it is possible that such units will be more precisely defined in the future in terms of one of the non-pigmented essential constituents, for example, an enzyme factor. Indeed, it is clear that Emerson (*80*) and Briggs (*53*) consider that a ratio such as 2,500/1 represents what happens by chance to be in a fully green cell the ratio of chlorophyll molecules to some such other factor in the hypothetical unit. Briggs (*54*) has

postulated that a substance A in the chloroplast (p. 238) is essential for photosynthesis. The amount of this substance present may determine the rate of photosynthesis. He reported that the ratio of A to chlorophyll in the experiments performed at Cambridge was of the order 1/500. Finally, we note that the kind of work that went on in Baas Becking's laboratory (p. 227) may possibly lead to useful developments of the idea of functioning pigmented granules composed of photosynthetic units.

Light and dark reactions. Recent work strongly suggests that the protoplasmic or enzymic factor can be resolved into several components. We already possess some evidence that carbohydrates are not produced from carbon dioxide and water by a single photochemical change. F. F. Blackman showed that when photosynthesis is proceeding relatively rapidly in well-illuminated leaves receiving an ample supply of carbon dioxide, Q_{10} is always greater than 2.[1] He therefore suggested that in addition to at least one photochemical stage in photosynthesis there must be at least one ordinary chemical stage. This so-called dark stage he attributed to the activity of a protoplasmic factor. From the results of his experiments (see p. 244) on the influence exerted by cyanide on the rate of photosynthesis by Chlorella, Warburg inferred the existence of a dark stage, which he described as *the Blackman reaction.* Since it was well known that cyanide exerts a strong depressant action on catalase, he suggested that the Blackman reaction might consist in the decomposition of a peroxide by an enzyme that had similar properties to those of catalase. More recently, evidence has been obtained that dark enzymic reactions occur at the outset of photosynthesis when CO_2 is absorbed as well as at the end when oxygen is liberated, and that the fixation of CO_2 may be inhibited by cyanide. There may therefore be two sets of Blackman reactions, *i.e.*, reactions having temperature coefficients greater than 2. Some writers suppose that the photochemical reaction, which may involve an oxidoreduction, occurs

[1] The temperature coefficient of a chemical reaction or of a physical process is the ratio of the rate of a reaction or process at a given temperature to the rate of the process at a temperature 10° C. lower. The symbol Q_{10} is conventionally used to represent this coefficient. For instance, if for any change a rate of x per unit time is obtained at 5° C., and $2x$ per unit time at 15° C., we may state that for this change $Q_{10} = 2$. It is well established that for purely photochemical reactions Q_{10} is usually less than 1·4, but that for ordinary chemical reactions Q_{10} is at least 2. Ballard (*12*) gives a list of Q_{10} values, obtained by various workers, for the chemical stage of real photosynthesis. Corrections were made for the Q_{10} values of the accompanying respiration. In some experiments on algæ and Angiospermic leaves these values have fluctuated between 1·5 and 2·5. In others much higher values have been obtained. For example, a value of 4·6 was found for Chlorella between 4° and 14° C. Using high light intensities and CO_2 concentrations greater than two per cent., Ballard himself obtained for the rate of apparent assimilation values of from 3 to 10 for castor-oil cotyledons, and a value of 4 for privet leaves, over the temperature range of 6° to 16° C. Ballard suggested an explanation for the high values he found (see p. 244).

between these two sets of dark reactions. For the remainder of this chapter the term Blackman reaction will refer to the dark reactions that consume the products of the light reaction.

We shall, below and later (pp. 246 to 258), record some evidence that CO_2 absorption and oxygen evolution in photosynthesis are distinct processes, the former being a combination with some organic substance in the chloroplast, and not a direct union with water as was at one time thought, and the latter taking place after water has entered into combination with the complex reacting system in the chloroplast. The oxygen evolved may come from water molecules, and not from those of carbon dioxide, after the formation of intermediate organic peroxides. The hydrogen atoms in these water molecules may be those which bring about the formation of carbohydrates by the reduction of carbon dioxide. This is not necessarily itself a photochemical reaction, but may be dependent upon the activity of a reducing agent which is only produced in the presence of light and of hydrogen atoms coming from water.

We are still, however, a long way from being able to write equations showing the actual molecules which participate in the light and dark reactions of photosynthesis. Many suggestions have been made by chemists (see Spoehr, *280*, Stiles, *287*, Emerson, *80*, and, for a summary of the formaldehyde hypothesis, Thomas, *308*), but there is little or no biochemical evidence to support any of them. For example, all attempts to prove that formaldehyde is an intermediate product in photosynthesis have so far been failures (see pp. 215 and 217). We do not know with what substance or substances in the chloroplast (chromoprotein, lipoid, etc.) carbon dioxide and water, probably independently of one another, first enter into combination; nor from what substance in the cell oxygen is split off; nor what substance is the immediate precursor of carbohydrate. Furthermore, only a beginning has been made in the analysis of the enzymes present in chloroplasts. Evidence of the presence of catalase and of oxidation enzymes should, however, be noted (p. 12). Clearly such evidence may throw light on the types of chemical reaction taking place in chloroplasts during photosynthesis, but it must be remembered that some of these enzymic changes may occur not in the actual production of carbohydrates but in some other protoplasmic process (*e.g.*, respiration, or the production of pigments).

It will be convenient at this stage to mention some of the biochemical evidence which has led to the conclusion that CO_2 absorption and oxygen production are distinct steps in the linked reactions which comprise photosynthesis. We shall then indicate the mode of analysis used by Briggs, which has greatly influenced thought in this country. Lastly we shall describe evidence of the occurrence of light and dark reactions in photosynthesis which has

come from the study of induction phases, and of photosynthesis in intermittent light.

(i.) *The absorption of CO_2 by green cells without the liberation of oxygen.* In 1939 it was reported by McAlister that certain green cells may continue to absorb carbon dioxide for a short time in the dark after a period of active photosynthesis in the light. No evidence was obtained that oxygen is produced under these conditions. Aufdemgarten made a similar report. Ruben *et al.*, using CO_2 with a radioactive carbon atom, secured evidence that green cells can absorb this gas in the dark and synthesize carboxylic groupings which contain some of the labelled carbon atoms. This synthesis, which is enzymic and sensitive to cyanide, may go on rather slowly for as long as an hour, and, as far as is known, does not proceed beyond the acid stage. The fact that many non-green cells can assimilate carbon dioxide to produce carboxylic acids is now well established (p. 339); indeed, Ruben showed that etiolated plants can do this. Although a linkage between carbon atoms is thus established, the carbon dioxide is not reduced, and the reaction is only weakly endothermic. It is evidently possible that some, at least, of the CO_2 uptake in the dark may be governed by an enzyme that is not a part of the photosynthesizing mechanism. This question is further discussed on p. 248. It may be that the CO_2 pick-up observed by McAlister and by Aufdemgarten was a different process from that studied by Ruben. Clearly our knowledge about this subject is not yet definite.

(ii.) *The liberation of oxygen by illuminated isolated chloroplasts and by intact cells without the absorption of CO_2.* Hill (*129*) separated chloroplasts from leaves of *Stellaria media* (p. 12), and showed that when they were freed from other cell components, supplied with carbon dioxide, and illuminated, they did not give off oxygen. When, however, certain ferric salts were added (*e.g.*, ferric oxalate) the illuminated chloroplasts gave off oxygen. This evolution happened even in the absence of carbon dioxide. Hill measured its rate by a delicate method which he devised (p. 222), and found that per unit mass of chlorophyll it was about one-tenth of that shown by a leaf photosynthesizing under favourable conditions. Leaf extracts that were free from ferric ions, but contained other ions (*e.g.*, those of calcium) and organic solutes, also induced the liberation of oxygen, but their action was not so powerful as that of ferric ions. Among the conclusions which may be drawn are (*a*) that the power of chloroplasts to photosynthesize carbohydrates *in vitro* is dependent upon some substance which is readily washed out of them, or is contained in cell materials outside them, and (*b*) that chloroplasts contain a mechanism whereby, under certain conditions, oxygen can be produced independently of the absorption of carbon dioxide.

Hill admitted that these isolated subcellular systems containing chlorophyll do not necessarily produce oxygen in a reaction identical with that which causes its evolution in normal photosynthesis, but Hill and Scarisbrick (*132*) found parallels in the two processes. They made a close study of the ferric oxalate reaction, measuring not only the oxygen output but also the amount of ferrous ions produced by the photoreaction. They showed that the chloroplast system in control of this oxidoreduction was thermolabile, and therefore presumably enzymic, and in some other respects resembled that part of the chloroplast system which *in vivo* controls the photochemical phase of photosynthesis. For example, it was inhibited by urethanes, but not by cyanide, which differentially poisons a dark reaction in photosynthesis.

We have no space to consider in detail Hill's and Hill and Scarisbrick's arguments concerning those reactions in chloroplasts, photosynthesizing *in vivo*, which may correspond to the ferric oxalate reactions in isolated chloroplasts. They assume that chloroplasts contain some substance, whose nature is unknown, which can, in the light, participate in oxidoreductions that lead to the evolution of oxygen. They concluded that "oxygen is itself formed in a photochemical reaction during which there is no reaction involving CO_2" and that "the light reaction in vegetable photosynthesis is the production of the oxygen molecule and is not the reduction of CO_2." Conceivably, however, the photochemical reaction they had in mind may take place in stages. Only one of the partial reactions may actually be light activated, and not necessarily the one by which oxygen is liberated (see also p. 252). Their experimental results, therefore, although not providing support for the view that the actual liberation of oxygen is a dark reaction which occurs subsequently to a photochemical reaction, are not decisively antagonistic to this view. In other respects their results lead to the following conclusions, which are similar to those tentatively drawn by certain other modern workers. The reaction in which oxygen is liberated is not directly linked with that by which CO_2 is absorbed. The oxygen set free does not come from CO_2 molecules, and may come from water, which is presumed to be a reactant in photosynthesis. There is at least one oxidoreduction process in photosynthesis, and this is in some way actuated by the light energy absorbed by chlorophyll.

Fan *et al.* (*84*) have reported that, by feeding illuminated Chlorella with certain reducible substances in the absence of CO_2, they caused this unicellular alga to give off appreciable quantities of oxygen. They satisfied themselves that the added substances did not cause the production of CO_2, which might have been immediately used in normal photosynthesis, before oxygen evolution was stimulated. Positive results were obtained with ferric salts (*cf.* Hill's experiments), and with acetaldehyde, benzaldehyde, and certain other organic compounds. No extra oxygen came off when formaldehyde, C_4 and C_6 acids, hexoses, phosphohexoses, and certain other compounds were added. For their experiments with benzaldehyde they suggested that the oxygen-liberating reaction may be represented thus:

$$2\ C_6H_5CHO + 2H_2O = 2\ C_6H_5CH_2OH + O_2$$

The equation implies that the oxygen given off comes from water. They claim that they have provided evidence of the evolution of oxygen *in intact cells* without simultaneous CO_2 reduction, and therefore support the view that CO_2 absorption and oxygen production occur in separate reactions.

(iii.) *Briggs's theoretical analyses.* In the light of growing knowledge about the kinetics of photosynthesis in green cells during

development and under varying external conditions, Briggs (*53* and *54*) has from time to time formulated in general terms schemata in which are taken into account the parts that may be played in chloroplastic activity by essential factors other than pigments. In all his papers he makes very guarded mention of reactants other than carbon dioxide, and of products other than carbohydrates and oxygen, but he indicates clearly in the equations in his schemata that dark chemical changes as well as a photochemical change occur on illuminated reactive pigmented surfaces of chloroplasts.

In one schema Briggs assumes that carbon dioxide combines with a substance S (which may be but is not necessarily chlorophyll) to give a complex S_c, which is then activated by light energy, yielding S_l.

$$S + CO_2 \rightleftarrows S_c \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$S_c + \text{energy} \rightleftarrows S_l \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

S_l may be regarded as the product of the photochemical stage.

Clearly the rate of formation of S_l would be governed by the concentrations of S and carbon dioxide and by the light intensity. It should be noted that the temperature coefficient of reaction (2) is unity.

Briggs postulates that the fate of S_l, however this photochemical product may be formed, depends upon the activity of a catalyst B. We may regard B as a protoplasmic factor in the chloroplast. In accordance with modern theories of the kinetics of enzyme action, Briggs suggests that B combines reversibly with its substrate S_l to give X, and then decomposes this compound with the production of carbohydrate and oxygen, and the regeneration of S and B.

$$S_l + B \rightleftarrows X \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$X = B + S + \text{products} \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

It should be carefully noted that Briggs postulates that reactions 1, 2 and 3 are reversible. This means that if the activity of B is insufficient immediately to convert S_l as in reactions 3 and 4, the excess of the photochemical product would be reconverted into S and carbon dioxide.

Equation 1 and 4 may be regarded as representing the principal dark changes in photosynthesis. Like other enzymic actions they may be strongly influenced by temperature ; they have Q_{10}'s greater than 2 (see above). Moreover, they may be differentially inhibited by cyanides and other poisons. It should be noted that the photochemical reaction (2) is influenced neither by temperature nor by cyanide.

In his most recent analysis Briggs postulates that there is present in the chloroplasts a substance A which is activated to produce A′ by the light energy absorbed by chlorophyll. According to this

hypothesis chlorophyll acts as a photosensitizer. (This would account for the fact that up to a certain limiting value, increases in light intensity can compensate for a deficiency in pigment.) *The production of A′ from A would then be the principal or possibly the only light reaction in photosynthesis.* He considered and did not reject the view that A′ may be a reducing agent, and that S_c with which A′ combines may be a hydrogen acceptor. He denoted by the symbol S_c' the substance, which may be a reduction product of S_c, formed by the combination of S_c and A′, and supposed that it gave rise to the products of photosynthesis. Inasmuch as Briggs regards the total process of photosynthesis as comprised of a series of linked light and dark reactions of which the first and last set are dark reactions, and at least one in between an oxidoreduction actuated directly or indirectly by absorbed light, his views are not different from those of certain other modern investigators (p. 251). In the remainder of this subsection and in the next one we shall, in order to simplify analysis, regard equation (2) as an overall one for all the reactions involving S_c, A, and light energy, and regard S_l as corresponding to S_c'.

(iv.) *Induction phases.* In 1918, Osterhout and Haas reported that although photosynthesis began at once when the marine alga Ulva was exposed to light after spending a period in the dark, the rate steadily increased until a constant speed was attained. The induction phase was over in about an hour. Later, Warburg observed a gradual increase in the photosynthetic activity of highly illuminated Chlorella cells in the first three minutes after transferring from the dark to the light. There did not appear to be an induction phase when weak illumination was used. Briggs (*52*) found that the rates of photosynthesis shown by leaves of the moss *Mnium undulatum* and of various angiosperms were at first slow on transferring from the dark to relatively high illumination, and subsequently rose until steady values were reached.

At constant temperatures the rise was greater in higher than in lower light intensities. Briggs interpreted in terms of his schemata his own results and those obtained by the other workers. A satisfactory explanation is that in the dark an inhibitor is formed which, like cyanide, interferes with the Blackman reaction, but not with the photochemical reaction. In the light this inhibiting action is destroyed. Clearly this destruction would lead to an increase in the rate of photosynthesis, provided the extent of the inhibition at transition had been sufficient to retard the rate of consumption of S_l, the photochemical product, under the light conditions of the experiment. At low light intensities S_l is produced at a relatively slow rate, and the activity of B, although presumably lessened by the presence of inhibitor, may still be sufficient to consume S_l at the

same rate as it would were no inhibitor present. Accordingly, at low light intensities there is less chance of the occurrence of an induction phase.

(v) *Photosynthesis in intermittent light.* In 1919 Warburg reported that the amount of *photosynthesis per second* (continuous rate) shown by cells of Chlorella exposed to continuous illumination was less than the amount of *photosynthesis per second of illumination* (intermittent rate) shown by cells exposed to rapidly alternating and equal periods of light and darkness. The intermittent rate was increased as the periods were shortened, and in some experiments it was nearly double the continuous rate. Evidently a given quantity of incident light energy was used in photosynthesis to a greater extent when supplied, not continuously, but in a succession of small amounts during short light periods with intervening dark periods. A possible explanation of this is that an essential phase of photosynthesis goes on independently of the presence of light. In intermittent light this phase would then proceed in the dark periods as well as in the light periods, and, consequently, the rate of photosynthesis per unit time of illumination would be enhanced. Emerson and Arnold (see Emerson, *81*, and Briggs, *53*) illuminated Chlorella suspensions in bicarbonate solutions with intense light emitted from neon tubes in flashes of 10^{-5} seconds duration, with intervening dark periods of varying lengths. Since each flash was so short we are probably justified in assuming that the amount of photosynthesis occurring during a light period was exceedingly small. Nevertheless, the rate of photosynthesis, measured for the whole period of an experiment with intermittent illumination, was not inconsiderable. Clearly this finding provides direct evidence that events concerned with photosynthesis occurred during the dark periods. Emerson and Arnold suggested that under the conditions of their experiments the Blackman reaction (Briggs's reaction 4) might have proceeded to completion during the dark periods, with the result that such of the photochemical primary product (Briggs's S_i or X) as was formed during a light flash was completely consumed. Briggs maintains, however, that some S_i may in the dark be reconverted into S and carbon dioxide. Emerson and Arnold obtained good evidence that, with the intense flashes they used, the process taking place in the dark had a high temperature coefficient. Their results suggest that the Blackman reaction gave the same yield in 0·03 seconds at 25° C. as it did in 0·4 seconds at 1° C. This work represents the first attempt to measure the rate of the dark phase in photosynthesis as an event apart from the whole process.

C. The Rate of Photosynthesis

Inasmuch as the rate of photosynthesis under constant external conditions of CO_2 concentration, light intensity, and temperature,

may increase during the development of a green leaf we may infer that the rate of the whole process is governed by the activity of *internal factors.* These are presumably operative within the chloroplasts (p. 231) and may, possibly, be organized within pigmented granules into photosynthetic units (p. 233). We have already cited clear evidence that the activity of an enzyme rather than the concentration of chlorophyll may frequently determine the rate of photosynthesis under constant external conditions. For green leaves that are developing under normal conditions it is not easy to obtain conclusive evidence that the rate of photosynthesis may be governed by the concentration of chlorophyll, since, as the leaves become greener, the activity of the enzyme factor may also be increasing. What is required as experimental material are comparable samples, taken from a given pure line of leaves or algal cells in which the protoplasmic factor is equally developed, but containing different concentrations of chlorophyll.

The mass of evidence seems to suggest that in fully green cells the concentration of chlorophyll is usually greatly in excess of that which can effectively operate with the other factor or factors in the chloroplast (*cf.* remarks above on the photosynthetic unit). Thus Willstätter and Stoll found that the leaves of ten-day-old runner-bean seedlings showed strong activity as soon as the first traces of chlorophyll were formed in them. Briggs (*49*) obtained evidence that twelve-day-old yellow-green leaves of this species photosynthesized at about the same rate as fully green leaves of the same age. It was presumed that the protoplasmic factor had developed to about the same extent in the two sets of leaves. These experiments were carried out at relatively high light intensities. It should be noted, however, that at low light intensities the photosynthetic activity of fully green leaves was greater than that of a comparable sample of green-yellow leaves. It appears, therefore, that to some extent a high light intensity may compensate for a shortage of chlorophyll. This fact supports Briggs's suggestion that chlorophyll may function as a photosensitizer (p. 239).

One of the major problems in plant physiology during the present century has been to determine how the rate of photosynthesis is governed by the *external factors*, CO_2 concentration, light intensity, and temperature. A few general statements will be made by way of introduction to other books and papers (Barton-Wright *17*, Briggs *49* and *50*, Spoehr *280*, Stiles *287*), in which it is related how an erroneous but fruitful hypothesis, viz., that of separate and independent governing factors, guided critical researches for nearly twenty years, and led to the present formulation of the problem in other terms.

In certain important investigations (James, *151*, Maskell, *189*) on the relation between the rate of photosynthesis and the *external*

concentration of carbon dioxide, special consideration has been given to the rate of diffusion of carbon dioxide from the environment to the chloroplast, as an operative factor. The diffusion phase in photosynthesis has been already discussed (chap. X), and it must suffice here to state that the rates of the anabolic events will obviously depend upon the rate at which one of the essential components is supplied. For instance, the rate of photosynthesis in the leaf of a land plant may be reduced by the narrowing of stomatal apertures. Such narrowing becomes especially important as a factor controlling the rate of photosynthesis when the air that surrounds leaves contains a low concentration of carbon dioxide, as happens under natural conditions in the field. In experiments on land plants high concentrations (*e.g.*, five per cent.) have often been used. It should be noted that one advantage attaching to the use in experiments of algæ, water mosses, and other hydrophytes, is that these plants do not possess stomata.

If photosynthesis is a complex anabolic event comprised of linked light and dark biochemical reactions, the rate of the whole process will at any time be limited by the rate of the slowest constituent reaction. For example, if dark reactions (see equations (1), (3) and (4) above) follow a light reaction (see equation (2) above), the rate would be limited either by the rate at which the photochemical product (S_i of Briggs's schema) is formed by the chloroplast, or by the rate at which this product is further metabolized by the protoplasmic factor (B of Briggs's schema) in the chloroplast.

It is now well established that the rate at which the photochemical reaction proceeds in a given leaf is governed by the concentration of carbon dioxide at the chloroplast surfaces, and by the light energy incident upon these surfaces. The equations (1) and (2) are consistent with these experimentally determined facts. The CO_2 concentration, and the light intensity, although separate external factors, are not independent factors as was at one time thought. The inter-relation between these factors is shown in graphs in which the rates of photosynthesis in a given leaf kept at constant temperature, which was not " limiting ", but under varying light intensities are plotted against the CO_2 concentration. We see that in the experiments which gave the results represented as curves in fig. 31, the rate of photosynthesis was always increased either by increasing the external concentration of carbon dioxide or by increasing the light intensity. Neither CO_2 concentration nor light intensity was in itself a limiting factor, but each was, in a sense, a deficient factor. It should be noticed that the curves as drawn in fig. 31 gradually flatten as they rise. This representation indicates that as the CO_2 concentration was increased, light intensity gradually replaced it as the factor which was in *relative minimum*, and that there was no sudden

substitution of one limiting factor for another. This should be compared with the earlier view that the rate of photosynthesis at any instant is limited by a single factor. Those who held this view graphically represented the substitution of one limiting factor for another by drawing rising straight lines which suddenly became horizontal (see Stiles, *287*).

The rate of the late dark chemical phases will be governed (*a*) by the rate of the photochemical phase which precedes it, for this will determine the supply of the photochemical product, which provides S_l, the substrate for the dark chemical phase, and (*b*) by the activity of the protoplasmic factor B. We have already pointed out that

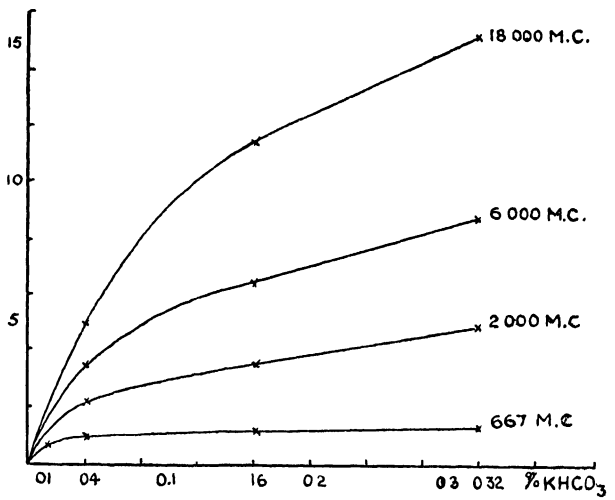


FIG. 31. The effects on the rate of photosynthesis of Fontinalis of changing the concentration of carbon dioxide at different light intensities. (From Harder, see Spoehr, *280*.)

this activity is not independent of external conditions. It fluctuates with the temperature, increasing in most leaves from low temperatures to some temperature between 30° C. and 40° C., and then decreasing as the protoplasm is progressively injured and killed during prolonged exposures to higher temperatures. For the process as a whole the connection between the light and dark phases on the one hand and the external factors on the other has been demonstrated in a variety of ways. For example, it has frequently been shown that photosynthesis is feeble at low temperatures in well-lit leaves plentifully supplied with carbon dioxide. We may infer that the rate of one of the later dark chemical reactions limits the rate of photosynthesis to this low value, *i.e.*, the protoplasmic factor (B) at this low temperature can only change a small proportion of the activated products (S_l)

of the photochemical phase. This inference receives further support from the fact that under these conditions the rate of photosynthesis is increased by raising the temperature, *i.e.*, by increasing the activity of the protoplasmic factor. We may state that under such conditions temperature is the external factor which is in relative minimum.

At this stage we may note that the effect of cyanide on photosynthesis (p. 234) appears to be similar to that caused by a reduction in temperature. Ignoring for the present the possibility that cyanide may retard or prevent the reaction by which S_c is produced, we note that if it combines with B, it will depress the activity of this catalytic system. In well-lit green cells S_l will be produced at a relatively fast rate ; and, even in the absence of cyanide, the activity of B may limit the rate of the whole process. The slightest depression of this activity, such as may be brought about by low concentrations of cyanide, would then immediately reduce the rate of photosynthesis. In his experiments (see also p. 234) Warburg found that the percentage reduction of rate was considerable when well-lit Chlorella cells were treated with weak cyanide. In cells under weak illumination the depression was negligible, presumably because the rate of production of S_l was so slow that the activity of B could, at the temperature of the experiment, be considerably reduced without affecting the rate of consumption of S_l in the Blackman reaction.

Ballard (*12*) has reported that in his experiments at 6° C. on castor-oil cotyledons and privet leaves concentrations of carbon dioxide above 2 to 2·5 per cent. acted as an inhibitor of the hydrogen cyanide type. The percentage inhibition was greater at high light intensities. It has long been known that at ordinary temperatures concentrations of CO_2 of from ten to twenty per cent. can depress photosynthesis. Ballard suggested that carbon dioxide, like HCN, may combine with the catalyst B, and so inhibit the breakdown of S_l. He pointed out that at high light intensities, which would promote its production, relatively more S_l would be prevented from yielding products of photosynthesis, and, consequently, the percentage depression measured would be greater. He accounted for the fact that for a given light intensity and concentration of CO_2 there was a greater depression at low temperatures than at ordinary temperatures by assuming " that the dissociation constant of the compound of carbon dioxide with the substance B increases with rise of temperature," and states that this assumption would also help to explain the high temperature coefficients which he obtained in his experiments (see footnote, p. 234). There would be a dual effect in raising the temperature from 6° to 16° C. The rates of the dark reactions for a given activity of B would tend to increase, presumably to the same extent as they would if the leaves were photosynthesizing in air. In addition, the activity of B would steadily increase as the depressant effect of CO_2 diminished.

It has long been known that photosynthesis is always feeble, (*a*) in poorly-lit leaves well supplied with carbon dioxide but not in inhibitory concentrations, and (*b*) in well-lit leaves placed in very low concentrations of carbon dioxide. Under these conditions the rate of the whole process is determined by the rate of the photochemical reaction and may be increased by increasing the light intensity in (*a*), and by increasing the concentration of carbon dioxide in (*b*). In both circumstances the photochemical reaction would be accelerated,

and the amount of activated substrate (S_f) offered to the protoplasmic factor (B) in unit time thereby increased. The protoplasmic factor will continue to be sufficiently active to deal at once with all the photochemical product, as long as the light intensity is deficient, under the conditions of (*a*), or the CO_2 concentration, under the conditions of (*b*), *i.e.*, the rate of photosynthesis will not be increased by raising the temperature until both the CO_2 concentration and the light intensity have been considerably raised. It should be carefully noted that, at low light intensities, the value of the temperature coefficient approaches unity.

Field experiments also show that CO_2 supply, light intensity, and temperature, may each in their turn limit the rate of photosynthesis between dawn and dusk. But it is not always easy to decide what factor is, at any time, relatively the most deficient. Sometimes, however, the situation is quite clear. Thus on warm days just after dawn or before dusk either in water plants or in land plants with open stomata, light energy is in relative minimum, *i.e.*, the rate of the photochemical phase limits the rate of the whole process. Then on bright winter days the rate in evergreen leaves is often limited by the temperature, *i.e.*, by the rate of the dark chemical phase. One of the many advantages accruing to plants from greenhouse culture during the winter is therefore not far to seek.

Since the CO_2 concentration in the air is low, and since stomatal apertures are not always fully open during the day, it is not surprising that the CO_2 supply often limits the rate of photosynthesis during the major part of a summer's day. The average daily rate of photosynthesis may be increased by raising the CO_2 concentration around a plant. This has been done for certain greenhouse crops, *e.g.*, tomatoes, and the yield from these plants has thereby been substantially increased.

Since over 90 per cent. of the dry matter of green plants is produced as a result of photosynthesis, it is clear that researches on the rate of photosynthesis are of great practical importance. Under cultural conditions for well-watered plants, an optimum balance between CO_2 concentration, light intensity, and temperature, should be the aim. These external factors may be readily controlled by mechanical means. The control of the activity of the internal factors is a problem for the plant breeder. Vigorous strains should be selected, and they should be grown under conditions which favour the development and maintenance of the full potential activity of the pigments and protoplasmic factors in the chloroplasts. It is well known that etiolated plants grown in the dark, or chlorotic plants grown in the absence of iron, lack chlorophyll. It follows that, in addition to a supply of the proper nutrients containing the elements found in the chlorophyll molecule (p. 176), light and soluble iron salts are necessary

factors for the formation of green pigments. Furthermore, Briggs (*50*) found that the protoplasmic factors do not develop full activity when the nutrient solution absorbed by the roots is deficient in certain essential elements, such as potassium, magnesium, iron, and phosphorus. These findings cannot be interpreted until we know the chemical nature of substances such as S, A, and B, which, according to Briggs, may be essential components of the photosynthesizing mechanism. Clearly the efficiency attained by a green leaf considered as an organ of photosynthesis will depend upon its nurture as well as its nature.

D. The Reduction of Carbon Dioxide by Cell Mechanisms Distinct in at least one respect from those Functioning in Normal Photosynthesis [1]

In some recent discussions on the mechanism of normal photosynthesis by green plants, much significance has been attached to the fact that carbon dioxide can be reduced, without the liberation of oxygen, by certain bacteria, and, under special conditions, by a few algæ. The bacteria multiply; accordingly for them the evidence is conclusive that organic substances are synthesized. The hypothesis which has prompted and is guiding these discussions is that the actual reduction of carbon dioxide may proceed along similar lines in all organisms capable of effecting it, although the chemical nature of the reducing system may be variable, and there may be diverse methods of raising a reducing potential to an effective level. Concede the hypothesis, then we may look to comparative biochemistry for a measure of elucidation of some of the problems discussed in this chapter. For example, we might deduce immediately (*a*) that the actual reduction of carbon dioxide is a dark reaction, and (*b*) that the production of oxygen is a feature peculiar to green plants, and a thing apart from the utilization of carbon dioxide. We have already considered these two points.

In describing the reactions in this section, we shall provisionally assume that the product formed by the reduction of carbon dioxide is always a carbohydrate, and shall adopt the modern convention of representing it by the non-committal formula (CH_2O). For every gram molecule of CO_2 assimilated in the production of one mole of (CH_2O), 112 kg. Cals. of free energy are required. In photosynthesis (and in what Gaffron calls photoreduction) this energy is provided by those light rays which are absorbed by normal chlorophyll or by bacterio-chlorophyll; in chemosynthesis it is provided by a collateral oxidation process.

[1] The reader will find in the recently published volume written by Rabinowitch (*242*) accounts of the subject matter of Sections D and E, and extensive discussions on the composition and structure of chloroplasts, and the biochemical mechanism of photosynthesis. These sections were written before the author received his copy of Rabinowitch's book.

Chemosynthesizing bacteria (see Stephenson, *282*). All the bacteria to be named in this subsection can develop in purely inorganic media,[1] using CO_2 or some form of carbonate as the sole source of carbon. They are aerobes and derive the energy used in synthesizing cell substances from the oxidation of some material, usually inorganic in nature, which is absorbed from the environment (see p. 261). Recent work has established that at least some of them (*e.g.*, *thiobacillus*) may show an endogeneous carbohydrate metabolism which is strictly comparable with that of yeast, the higher plants and tissues of higher animals. The exothermic oxidations for which equations are given below may therefore be plausibly regarded as mainly providing energy which is functionally comparable to the light energy that is absorbed by pigments in photosynthesizing cells.

(i.) *Nitrifying bacteria.* (See also p. 98.) Among the nitrifiers are *nitrosomonas*, which oxidizes ammonia with the production of nitrite,

$$NH_3 + 3O = HNO_2 + H_2O + 79 \text{ Cals.}$$

and *nitrobacter*, which oxidizes nitrite to nitrate.

$$HNO_2 + O = HNO_3 + 21{\cdot}6 \text{ Cals.}$$

(ii.) *Iron bacteria.* Living autotrophically in water containing ferrous ions, *Leptothrix ochraceae*, *Spirophyllum ferrugineum*, and other iron bacteria, oxidize ferrous ions to ferric ions, and ferric hydroxide may be precipitated. Natural waters in which such oxidations have occurred are often reddish in colour.

$$4FeCO_3 + O_2 + 6H_2O = 4Fe(OH)_3 + 4CO_2 + 81 \text{ Cals.}$$

(iii.) *Colourless sulphur bacteria.* Living in thermal springs or in stagnant water containing sulphuretted hydrogen there may be found certain autotrophic bacteria (*e.g.*, *beggiatoa*, *thiothrix*) which obtain their energy for chemosynthesis by oxidizing this gas with the intercellular deposition of sulphur.

$$H_2S + O = H_2O + S + 32{\cdot}5 \text{ Cals.}$$

When the H_2S supply is exhausted the sulphur deposit is consumed.

$$S + 3O + H_2O = H_2SO_4 + 141{\cdot}8 \text{ Cals.}$$

Thiobacillus thio-oxidans was discovered in soils containing free sulphur and rock phosphate. It differs from *beggiatoa* and *thiothrix* in being able to use an external source of sulphur or of thiosulphates. While chemosynthesis is in progress sulphuric acid is produced. The organism can tolerate concentrations as high as five per cent. of this acid.

$$S + 3O + H_2O = H_2SO_4 + 141{\cdot}8 \text{ Cals.}$$

Vogler *et al.* (*344*) at the University of Wisconsin have reported that the solid sulphur is dissolved in fat globules at the ends of cells, and that the oxidation takes place inside the cells. They state that the oxidation is inhibited by hydrogen cyanide and other poisons that are known to combine with oxidases containing iron or other metals. It has been established at Wisconsin that this bacillus contains phosphorylated carbohydrate esters of the kind found in other plants and in animal cells, and that it displays

[1] It is not surprising (see p. 380) that traces of certain special accessory substances have occasionally to be supplied with the inorganic nutrients. A single example must suffice. *Thiobacillus thio-oxidans* is regarded as an autotrophic organism because it can manufacture its own foods, later to be consumed in body building and respiration, from CO_2 and inorganic salts. Nevertheless, for it to grow traces of the following substances may have to be added to the culture medium : thiamin, pyridoxin, biotin, nicotinic acid, pantothenic acid, riboflavin.

endogenous respiration and fermentation under conditions (*e.g.*, in the absence of sulphur or of oxygen) which prevent the masking of these processes by chemosynthesis. At least for this organism the distinction drawn some years ago (see p. 261) between the energy for chemosynthesis and the energy for life appears to have been justified.

During active growth the amount of CO_2 absorbed is related to the amount of sulphur oxidized ; but Vogler has reported that a limited amount of CO_2 can be fixed by the bacillus, aerobically or anaerobically, even in the absence of oxidizable sulphur. It is of great importance to realize that such CO_2 assimilation does not necessarily mean the formation of a reduced product. For example on p. 338 we shall describe modern work which proves that the assimilation of CO_2 may result in the formation of a carboxylic group. The term chemosynthesis, like the term photosynthesis, implies a reduction of carbon dioxide in a strongly endothermic reaction. Carboxylation is not such a reduction, for, if endothermic, it is only weakly so. It may possibly be that there is a danger of some confusion in thought because full cognizance has not been taken of the fundamental difference between reduction and carboxylation. Nevertheless the idea cannot be rejected that the *uptake* of CO_2 in the absence of sulphur is identical with that occurring in the presence of sulphur, for this uptake might be the step preliminary to reduction which, as a strongly endothermic reaction, can only proceed when sulphur is being oxidized.

It has further been shown at Wisconsin that when *thiobacillus* is oxidizing sulphur, inorganic phosphate is absorbed by the cells. This absorption is particularly vigorous in the absence of CO_2. If cells which have been living in media containing sulphur and in the absence of CO_2 are transferred to sulphur-free media and are supplied with CO_2, there is a strong assimilation of this gas, and inorganic phosphate is released from the cells to the media. Vogler and Umbreit have put forward the important suggestion that some of the energy released in the oxidation of sulphur may, in the absence of CO_2, become incorporated in active phosphate bonds contained in such phosphate esters as are produced in a coupled reaction, and that later this phosphate-bond energy may be used in CO_2 assimilation in the absence of sulphur. On the basis of this notion Vogler has posed the question, "Is it possible to irradiate photosynthetic organisms in the absence of CO_2 and to store at least a portion of the radiant energy within the cell in a form which can later be used for CO_2 fixation in the dark ?" Emerson *et al.* (*343*), who belong to the Wisconsin circle, have reported their inconclusive results, obtained in experiments which were designed to answer this question. The problem is well stated in the introduction to their paper. The whole question is still *sub judice*. Even if we admit that CO_2 fixation in the absence of sulphur by *thiobacillus* is in some way coupled with the phosphatase hydrolysis of ester phosphate, we shall require evidence that carbohydrates or products of similar high energy level are produced as a result of the fixations that have been studied at Wisconsin.

(iv) *Hydrogen bacteria.*[1] Among the many bacteria which normally live heterotrophically in the soil there are some forms, *e.g.*, *Bacillus panto-*

[1] It should be noted that free gaseous hydrogen is not present in any environment where existing organisms grow. The power to oxidize hydrogen may be a purely accidental biochemical attribute without any historical significance. But inasmuch as in those primeval epochs when things able to grow and multiply first came into existence, free molecular hydrogen may possibly have been present in the atmosphere, there is scope for speculative argument that the power to consume hydrogen may have had functional significance in some primitive ancestors of the existing hydrogen bacteria.

trophus, which can be cultured in artificial inorganic media, provided they are supplied with hydrogen, oxygen, and carbon dioxide. Ruhland called them *Knallgas* bacteria (*i.e.*, detonating gas bacteria), because they derive their energy for the chemosynthesis of organic matter from the oxyhydrogen reaction

$$O_2 + 2H_2 = 2H_2O + 137 \text{ Cals.}$$

Ruhland found that when the form *B. pycnoticus* was growing rapidly, the value of the quotient *volume of hydrogen absorbed/volume of oxygen absorbed* was considerably greater than 2, and he made the important suggestion that the hydrogen consumed in excess of that represented by the above equation was used in the reduction of CO_2 to form cell materials.

$$2H_2 + CO_2 = (CH_2O) + H_2O - 112 \text{ Cals.}$$

He suggested that this endothermic reaction is coupled with the oxyhydrogen reaction. This suggestion implies that the hydrogen, or the CO_2, or both, are activated before the chemoreduction can be effected. If enzymes are concerned with the transfer of hydrogen to the CO_2, the production of carbohydrate by hydrogen bacteria may proceed along lines similar to those it follows in green photosynthesizing cells. Van Niel considers that water is the hydrogen donator in photosynthesis and that oxygen is ultimately produced from the hydroxyl part of the water molecule. Light energy absorbed by chlorophyll brings about such activations as must occur before CO_2 can be reduced by the hydrogen transferred through intermediary enzymes from water. Many of these points are still highly controversial, but a good case has been made for the thesis that the study of any system which occasions the production of organic matter by the reduction of CO_2 may help to elucidate the equivalent process which occurs as the climax of normal photosynthesis.

Photosynthesizing bacteria (see van Niel, *211*). It is certainly an attractive hypothesis that the reduction of carbon dioxide by such bacteria as can absorb light is biochemically similar in more than one respect to that occurring in normal photosynthesis.

Red sulphur bacteria (Thiorhodaceae) and purple non-sulphur bacteria (Athiorhodaceae) are facultative anaerobes, which contain carotinoid pigments and bacteriochlorophyll. For the synthesis of cell materials they require the light energy that is absorbed by their chlorophyll, and not that absorbed by the carotinoid pigments. There exist also green sulphur bacteria, but less is known about their metabolism. The molecular structure of bacteriochlorophyll is slightly different from that of the chlorophylls of algæ and other green plants, and these coloured bacteria can absorb rays in the infra-red region of the spectrum of wavelengths as high as 900 $\mu\mu$.

Receiving such rays in what appears to man to be the dark, the Thiorhodaceae live autotrophically and grow in inorganic culture media under anaerobic conditions if they are supplied with carbon dioxide as the sole source of carbon, and sulphur, hydrogen sulphide, or certain other inorganic sulphur compounds. Some of them can use molecular hydrogen and certain organic hydrogen donators. The simplest case to consider is that in which sulphur bacteria consume photobiochemically H_2S simultaneously with CO_2. Van Niel could

not demonstrate CO_2 disappearance in excess of that accounted for by the equation

$$2CO_2 + 2H_2O + H_2S = 2(CH_2O) + H_2SO_4$$

This equation epitomizes van Niel's view that the continuous production of carbohydrate is a light-activated oxidoreduction which involves a continuous supply of CO_2 and H_2S or some other reducer. Among the external factors which may limit the rate of photosynthesis are the supply of CO_2 and reducing agent, the light intensity and temperature. Among the internal factors are the concentration and activity of the participating enzymes and the amount and state of bacteriochlorophyll. There is good evidence that there are light and dark stages. The equation written above represents the overall reaction.

The metabolic function of H_2S in photosynthesizing sulphur bacteria is different from that of this compound in chemosynthesizing bacteria. In the former, which are active anaerobically, H_2S acts as a hydrogen donator, and it is the absorbed light energy which activates the molecules concerned in the reduction of carbon dioxide. In the latter, which are obligate aerobes, H_2S is oxidized by the absorbed oxygen, and a fraction of the energy liberated is used in the chemoreduction of carbon dioxide.

The following equation has been proposed for the anaerobic photosynthesis of cell materials from carbon dioxide, when hydrogen gas is supplied instead of H_2S as the reducing agent:

$$CO_2 + 2H_2 = (CH_2O) + H_2O$$

This equation is identical with that written on p. 249 for the aerobic chemosynthesis of carbohydrate by the colourless hydrogen bacteria. The catalytic mechanisms concerned with the two reductions are not necessarily identical, but it is a good working hypothesis that they may not be very different from one another. In the red bacteria the oxidoreduction, which may proceed through the agency of more than one hydrogen-transferring enzyme, is activated by light. In the colourless hydrogen bacteria it is activated by the energy derived from the aerobic oxidation of hydrogen. We note, therefore, that hydrogen serves one function in the red bacteria and, since it is an oxidizable substrate as well as a reducing agent, two functions in the colourless bacteria.

Bacteria belonging to the Athiorhodaceae, when they receive the proper red and infra-red rays, grow under anaerobic conditions, but require certain kinds of organic compounds as well as CO_2 and inorganic salts. They cannot use sulphur compounds as hydrogen donators. The organic matter may function in several ways. The function that interests us here is that of supplying in light activated cell systems the hydrogen required for the reduction of carbon

dioxide. Some of these bacteria can live aerobically in the dark. It is noteworthy that, when showing this alternative mode of life, they consume the same hydrogen donators as those used in the reduction of CO_2 in the light. In the aerobic dark process, however, oxygen and not CO_2 acts as the hydrogen acceptor. Evidence exists therefore that catalytic systems concerned with aerobic respiration may possibly participate in some forms of photosynthesis.

In the Athiorhodaceae aliphatic acids are very effective hydrogen donators, but, as they and their oxidation products are themselves consumed in other ways, they are not the best substrates to use in quantitative studies on the mechanism of these coupled oxido-reductions. It has been shown, however, that in certain strains of purple bacteria which have been isolated from soil isopropyl alcohol can serve as the hydrogen donator in the light-activated reaction that proceeds according to the equation

$$CO_2 + 2(CH_3)_2.CHOH = (CH_2O) + 2(CH_3)_2.CO + 2H_2O$$

Since the acetone produced is not metabolized further, it accumulates. When the isopropyl alcohol has been completely consumed the reduction of CO_2 ceases.

In all these sections on bacterial chemosynthesis and photosynthesis we have kept in mind that our main interest in this chapter is in normal photosynthesis by green plants. In 1926 Kluyver suggested that in such plants water is the specific hydrogen donor and, in illuminated cells, occasions the reduction of carbon dioxide according to the overall equation

$$CO_2 + 2H_2O = (CH_2O) + H_2O + O_2$$

Franck and Gaffron (*91*) consider that the insertion in this equation of a second molecule of water, "apparently introduced *ad hoc*, marks the turning point between the old conception of photosynthesis as a unique process of carbon dioxide decomposition by light and the modern idea that it should be looked upon as one of a group of similar oxidoreduction processes, all following the general formula."

$$CO_2 + 2H_2A = (CH_2O) + 2A + H_2O$$

It was van Niel who, adopting Kluyver's view for normal photosynthesis, proposed the above general formulation for all forms of photoreduction. The same equation can be made to serve for chemoreductions also. Van Niel strongly emphasized the fact that there exists no convincing evidence of the production of oxygen in any of the forms of bacterial photosynthesis which have been studied. In these when, for example, H_2A is H_2S or isopropyl alcohol, A will be respectively sulphur or acetone; when molecular hydrogen is the final reducer, A has no existence. According to van Niel it is a unique feature of normal photosynthesis for water to be the final hydrogen

donator: oxygen production, which distinguishes such photosynthesis from all other forms of overall reactions concerned with CO_2 reduction, is a consequence of this feature. When two molecules of water are dehydrogenated there will be left two hydroxyl groups, which, it is supposed, may unite to form a peroxide complex in the chloroplasts. There is no evidence that such peroxides ever accumulate during photosynthesis, but that may be because of the high velocity with which they are decomposed into the original peroxide-forming hydroxyl acceptors and molecular oxygen.

E. Normal Photosynthesis, Anaerobic Photosynthesis (Photoreduction), and Aerobic Chemosynthesis by Scenedesmus and certain other Green Algæ

Gaffron (see *94* for a review and bibliography) has discovered that Scenedesmus and certain other green algæ (*e.g.*, Rhaphidium, Ankistrodesmus), which show normal photosynthesis when illuminated under natural conditions, can be "adapted" by special anaerobic treatment to reduce carbon dioxide without the elimination of oxygen.

In the adapted algæ, as in some of the photosynthesizing bacteria, reduction can be effected anaerobically by molecular hydrogen in the light (Gaffron calls this photoreduction), and, as in the hydrogen bacteria, chemoreduction can be driven in the dark by the energy coming from the oxyhydrogen reaction. We have seen no statement that the algæ multiply under these abnormal conditions. One of Gaffron's main achievements is that he has advanced our knowledge of normal photosynthesis by making certain chloroplasts, which appear to act under aerobic conditions in the light like those of all other green plants, perform their functions of reducing CO_2 as a phenomenon apart from their function of occasioning the liberation of oxygen. Evidence has already been given on p. 236 that the evolution of oxygen and the reduction of CO_2 are controlled by catalytic systems that are not necessarily directly linked. Moreover, if, as Gaffron contends, the chemoreduction is under the same enzymic control as the photoreduction, although differently driven, it follows that the assimilation and the reduction of carbon dioxide in normal photosynthesis are both dark reactions. There is a possible conclusion that can be drawn from the fact that photoreduction by illuminated chloroplasts of adapted Scenedesmus occurs without the evolution of oxygen. It is that the actual enzymic decomposition which leads to oxygen production in normal photosynthesis may, like the actual reduction of CO_2, be a dark reaction (see also p. 237). Hydrogen adaptation may lead to a temporary inactivation of the enzyme concerned with this dark reaction, assuming it to be such. This evidence alone does not provide rigid proof that it is a dark reaction,

since the actual evolution of oxygen might require the combined action of an active enzyme and light energy. Obviously, if the enzyme is inactivated in the adaptation process, mere illumination would be insufficient to cause oxygen production, unless it made the enzyme active again: activity is not restored under conditions leading to photoreduction.

If we tentatively take up the position that the only light reactions in normal photosynthesis are those which occur in photoreduction, a plausible hypothesis can be put forward to cover what happens in both of these processes. As we have pointed out there is evidence that the actual reduction of CO_2, or of some compound of CO_2 (Briggs's S_c, see p. 238) with cell substance, is a dark process. Let us suppose that it requires the continuous supply of some effective hydrogen donator AH_2, acting alone or in conjunction with a dehydrogenase enzyme. It may be one of the pigments or one of the many other substances which are present in chloroplasts. The essential requirement is that its reduction potential should be high. Once formed, whether in photosynthesis, photoreduction, or chemoreduction, this effective hydrogen donator might bring about the reduction of CO_2, quite independently of light, according to the equation

$$CO_2 + 2AH_2 = (CH_2O) + H_2O + 2A$$

Clearly, for this reaction to go on continuously there must be another reducing mechanism in the green cells by which AH_2 is continuously regenerated from the oxidation product A. In normal photosynthesis by any green cell water may be the substance which acts as the ultimate hydrogen donator for the regeneration of AH_2. Ignoring some conjectures concerning intermediate stages, we may write the following overall equation for this regeneration:

$$2A + 2H_2O + \text{catalyst} \xrightarrow{\text{light energy}} 2AH_2 + \text{catalyst peroxide}.$$

It is an acceptable hypothesis that this reaction is endothermic, and that it is driven by the light energy absorbed by the green pigments in the chloroplasts. This would make it the light reaction in photosynthesis. It has been suggested that the peroxide is decomposed with the evolution of oxygen in a dark reaction by an enzyme which resembles but is not identical with catalase.

$$\text{Catalyst}\begin{matrix}\diagup \text{O} \\ | \\ \diagdown \text{O}\end{matrix} \xrightarrow{\text{enzyme}} \text{catalyst} + O_2.$$

The overall equation for photosynthesis constructed from the three partial equations given above would be

$$CO_2 + H_2O = (CH_2O) + O_2.$$

At least this scheme satisfies the essential requirement of accounting for the well-established fact that the photosynthetic quotient is unity.

At this stage it would be possible, but we think premature, to analyze in the mode of Briggs (see p. 235) how the rate of production of S_c, regarded as the substance reduced by AH_2, may limit the rate of photosynthesis, for example, when at high light intensities CO_2 is in short supply or when the temperature is low, and how the rate of photosynthesis may be limited by that of the production of AH_2 at low light intensities.

We must remember that photoreduction in adapted Scenedesmus occurs in an atmosphere of hydrogen. Gaffron believes that only such algæ can carry out photoreduction as are capable, under anaerobic conditions, of developing a hydrogenase enzyme, or of making active a hydrogenase already present in an inactive form. For example, Scenedesmus possesses this power, Chlorella lacks it. Under the agency of the hydrogenase, in adapted Scenedesmus, hydrogen is absorbed and reduces certain hydrogen acceptors. Gaffron has obtained evidence that some of these reductions can occur in the dark. Since photoreduction cannot, it is possible that the light reaction in this process consists in raising the reduction potentials of the hydrogen donators produced under the agency of hydrogenase. However this may be, the main trend of informed speculation appears to be in the direction of assigning the light reactions in photoreduction and photosynthesis to systems which control hydrogen transfers from donators to the reducer AH_2 which finally effects the dark reduction of CO_2 or S_c.

After this general introduction and indulgence in conjectures, based on a selected few of the wide-ranging ideas put forward by Briggs (*54*), Gaffron (*94*), Franck and Gaffron (*91*), and van Niel (*211*), some of the facts that Gaffron has discovered will be briefly described.

The adaptation reaction. The adaptation reaction may take several hours. It proceeds relatively fast when cells of Scenedesmus, suspended in a bicarbonate or phosphate buffer solution, are incubated in the dark in pure hydrogen, or in a gas mixture of hydrogen and CO_2. During adaptation hydrogen is absorbed, and the rate of absorption is a measure of the rate of adaptation. The reaction can also take place in pure nitrogen or in a gas mixture of nitrogen and CO_2: under these conditions the algæ eventually begin to liberate hydrogen from an unknown source by their anaerobic catabolism, and later to reabsorb this liberated hydrogen.

If Scenedesmus cells that have been kept in pure nitrogen in the dark until they begin to give off hydrogen are illuminated in the absence of CO_2, the hydrogen output is greatly increased. This photochemical elimination of hydrogen, which has not been observed in any other organisms, besides those mentioned at the beginning of this section, does not occur in the presence of CO_2. Gaffron has inferred that it results from the photodecomposition of internal hydrogen donors, which, in the presence of CO_2, hand on their hydrogen directly or through intermediary mechanisms to this gas. The evidence suggested to Gaffron that when hydrogen adaptation is completed there is present in the cells a hydrogenase enzyme

which controls the absorption of hydrogen and its donation to acceptor substances (A). The AH_2 substances so produced may then, when activated by light, participate in the reduction of CO_2.

Photoreduction and the turn-back reaction to photosynthesis. The algæ are said to be adapted for anaerobic photosynthesis when, on being illuminated in a gas mixture containing hydrogen and CO_2, they simultaneously absorb both of these gases. At a given temperature and light intensity the amount of CO_2 present in the gas mixture, or as bicarbonate in the liquid medium, determines the rate at which hydrogen is absorbed. Gaffron has stated

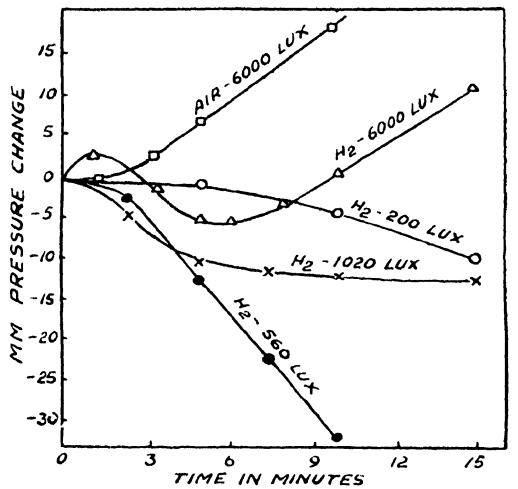


FIG. 32. Photosynthesis in air, and photoreduction and the turn-back reaction in hydrogen, by illuminated Scenedesmus (see text). From Franck and Gaffron (*91*) : Gaffron's experiment.

that the values he obtained for the relative rates at which CO_2 and hydrogen are absorbed were in accord with the following overall equation :

$$CO_2 + 2H_2 = (CH_2O) + H_2O$$

This same equation summarizes one form of photosynthesis shown by purple bacteria (p. 250).

As we have pointed out above Gaffron contends that the carbon dioxide which is assimilated by illuminated Scenedesmus under anaerobic conditions undergoes the same reductive changes in what he terms photoreduction as those which lead to its conversion into carbohydrate in normal photosynthesis by this alga. He maintains that the major difference between photoreduction and photosynthesis lies in the fact that whereas in the latter, light activated chloroplasts employ water as the hydrogen donator with the liberation of oxygen as a consequent but distinct phenomenon, in the former it is the hydrogen donators that are produced when hydrogen is absorbed during the adaptation reaction, and subsequently, which are used by the light activated chloroplasts to reduce carbon dioxide.

From data obtained by studying the influence of light intensity upon the changes in gas pressure which occur when hydrogen adapted Scenedesmus, suspended in a medium containing CO_2, is illuminated in hydrogen gas (fig. 32), Gaffron educed evidence that a substantial part of the mechanism

which performs photoreduction functions also in photosynthesis. First we note the increase in pressure brought about by the normal photosynthesis of the algal cells illuminated in air. This may be attributed to the production of insoluble oxygen in exchange for the dissolved CO_2 which the cells absorb. The form of the curve suggests that there was an induction period lasting about five minutes. Next we observe that when the alga was illuminated with 200 lux in an atmosphere of hydrogen the gas pressure decreased. This was owing to the absorption of hydrogen as well as CO_2 without the evolution of any gas. Gaffron found that the rate of hydrogen absorption, *i.e.*, of photoreduction, became faster as the illumination was increased up to a value of about 560 lux, but with further increases in light intensity there occurred sooner or later a turn-back reaction to normal photosynthesis with the evolution of oxygen. In general he concluded that the phenomenon of photoreduction can be demonstrated only in such low light intensities as will limit the rate of normal photosynthesis in the same cells. It is clear from the form of the curves that the turn-back reaction took place sooner at the light intensity of 6,000 lux than at that of 1,020 lux.

Gaffron has contended that the mechanism effecting the fixation and reduction of CO_2 in normal photosynthesis retains its activity in the chloroplasts of the hydrogen adapted cells, and is responsible for photoreduction. To effect continuous reduction, however, the mechanism in the reduced state must be continuously regenerated, which means that it must be coupled with another reducing system. In photosynthesis this additional system is the one which transfers hydrogen from water (p. 251) with the consequent evolution of oxygen. It is the only additional system available when cells are living in air, and gradually becomes the dominating one when hydrogen adapted algæ are exposed to high light intensities. But at low light intensities in such adapted algæ there is the additional system worked by a hydrogenase which is continually absorbing hydrogen and regenerating a reducing mechanism. Gaffron evidently regards the light reaction in photosynthesis and photoreduction as concerned, not with the actual reduction of CO_2, but with the intermediate transfer of hydrogen.

The chemoreduction of CO_2 in the dark, driven by the oxyhydrogen reaction. The presence of a trace of oxygen prevents the hydrogen adaptation of Scenedesmus. Even under low light intensities, cells of this alga show normal photosynthesis and no photoreduction when placed in gas mixtures containing hydrogen, carbon dioxide, and a trace of oxygen. After hydrogen adaptation has been completed, however, the temporary presence of measurable quantities of oxygen in the atmosphere in a closed vessel containing the algæ does not inhibit photoreduction because the cells perform the oxyhydrogen reaction (p. 249) so fast that the oxygen is quickly consumed. Gaffron discovered that cells placed in the dark in a gas mixture of hydrogen, carbon dioxide, and oxygen, absorbed all the three gases. He inferred that the CO_2 was reduced by the molecular hydrogen under the agency of enzymes, and that the reduction processes were driven by the energy coming from the oxyhydrogen reaction. The amount of oxygen consumed in ordinary respiration was negligible. This was the first time that the *Knallgas* reaction (p. 249) had been observed in green plants. Gaffron considers that photoreduction and the dark reduction of CO_2 in Scenedesmus proceed along the same pathway, but remarks that it is premature to state that the mechanism is identical with that which operates in chemosynthesis by the hydrogen bacteria (p. 249). Nevertheless there are now stronger reasons than any that could have been advanced heretofore for regarding information obtained about

bacterial chemosynthesis as suggesting what may happen in norma photosynthesis.

The differential inhibition of enzymes in chloroplasts. Gaffron has pointec out that whereas selective poisoning (what we have called differentia inhibition, p. 215) has yielded good results in the study of intermediat respiratory and fermentative metabolism, no accumulation of inter mediates and no deviation in chemistry have been detected by treatin illuminated green cells with hydrogen cyanide, carbon monoxide, hydroxyl amine, and so forth. Nevertheless, as we have seen (p. 234), Warbur found that the dark reaction in photosynthesis is more sensitive to cyanid than is the light reaction, and Ballard proved that this also holds good fo the inhibitory action of CO_2 in high concentrations. Gaffron has made us of several poisons in order to study in isolation the light and dark processe of normal photosynthesis and of photoreduction in hydrogen adapte algæ, the adaptation reaction, and the turn-back reaction. The essentia point to note is that these processes have been effected as events apar from one another. This affords hope that more information about thei chemical nature may be forthcoming in the next phase of the research.

We shall write a few brief notes to indicate the kind of observatio Gaffron has made and to state some of the conclusions he has drawn.

The CO_2-fixation reaction in photosynthesis and photoreduction i retarded by hydrogen cyanide, the percentage inhibition of photosynthesi being much greater at higher light intensities (p. 244). Adaptation i hydrogen with the acquisition of photoreductive power is, however, th most sensitive to cyanide of all the reactions. This poison appears t inhibit the set up of that reducing system in the chloroplasts which control the uptake of hydrogen. It may possibly combine with the precursor o hydrogenase. The turn-back reaction at high light intensities fron photoreduction to photosynthesis is accelerated by cyanide: this i understandable if the hydrogenase on which photoreduction depends i preferentially attacked by the cyanide. In chemoreduction such lo cyanide concentrations as do not appreciably affect either photosynthesi or photoreduction in Scenedesmus inhibit the coupling of the CO_2 reductio process with the oxyhydrogen reaction.

Hydroxylamine in low concentrations inhibits photosynthesis. It i equally effective at high and low light intensities, *i.e.*, whether the dark o light reactions are limiting the rate of the total process. Gaffron state that this poison does not prevent the reduction of CO_2, but inhibits specifi cally the liberation of oxygen. Purified catalase is poisoned by hydroxyl amine, but Gaffron does not regard the chloroplast enzyme as a tru catalase. He states, however, that it may have a similar constitution an properties. Conceivably it contains a ketonic or aldehydic group wit which hydroxylamine will combine.

Photoreduction by hydrogen adapted algæ is much less affected b hydroxylamine than is photosynthesis, but the process of hydroge adaptation is retarded by this poison, possibly because it tends to preven the production of a hydrogenase in its reduced form. Accordingly, i Scenedesmus cells are treated with hydroxylamine while they are in ai and if these cells are subsequently placed in hydrogen, photoreduction i very slow; but if the same hydroxylamine treatment is given after period of hydrogen adaptation, there is little effect on photoreduction.

We have seen that at high light intensities, *e.g.*, 6,000 lux, there is quick turn back from photoreduction to photosynthesis in hydroge adapted Scenedesmus. In the presence of M/1,000 hydroxylamine however, photoreduction will continue even at 6,000 lux. This is under

standable if hydroxylamine is poisoning one of the enzymes that is concerned with the utilization of water as a hydrogen donator in normal photosynthesis. Oxygen will not be produced, and the hydrogenase will not be oxidized. The turn-back reaction will not take place, and photoreduction will continue to occur.

Carbon monoxide has no effect on photosynthesis, but, since it inhibits the hydrogenase system, it interferes with all phases of the hydrogen metabolism of Scenedesmus.

So far no intermediate metabolites or derived products have been detected as accumulating as a result of any of the various forms of differential inhibition of the enzymes contained in chloroplasts which we have just described. It must be remembered that some at least of the reactions are reversible. Accordingly if one of the forward reactions in the direction of carbohydrate production or oxygen liberation is inhibited, back reactions may occur with the release of the carbon dioxide taken up in the first phase of photosynthesis, photoreduction, or chemoreduction. It will be recalled that Briggs emphasized the importance of these back reactions by representing as reversible reactions some of the stages postulated in his schemata.

CHAPTER XIV

RESPIRATION [1]

A. Aerobic Respiration as the Oxidative Consumption of Respirable Substrates with the Liberation of Free Energy and Heat Energy

EXPERIMENTAL work in the late eighteenth and early nineteenth century (see Sachs, *256*) established the facts about the gaseous exchanges between plants and the environment that were summarized in chap. X, section A. In the same chapter consideration was also given to the question of the paths along which gases diffuse into, within, and out of a plant. It was Sachs (1868) who first made it clear that plant respiration is quite distinct from photosynthesis, and often consists, as in animals, in (*a*) the continual absorption of oxygen, (*b*) the oxidation of organic substances contained in the respiring cells with a concomitant liberation of energy, (*c*) the liberation of carbon dioxide and the formation of water, and (*d*) loss in dry weight.

Because they will not grow in the absence of oxygen, green plants, parasitic and saprophytic higher plants, most fungi, and many bacteria are classed as aerobes. Among the phenomena that may be dependent upon the presence of oxygen are nuclear division, the power of movement, the absorption and migration of solutes, and the ability to secrete liquids. It is generally accepted that this oxygen need of aerobes is related to their energy requirement. In the absence of oxygen most aerobes produce carbon dioxide and liberate energy. But the energy set free by anaerobic respiration is either insufficient in amount or not released in the requisite manner for the occurrence of phenomena such as those mentioned above, and, consequently, for growth to take place. This energy is derived, not from the oxygen that is absorbed, but from certain of the anabolic products (termed respirable substrates) contained in the cells of the respiring tissue. In the presence of oxygen the protoplasm in the respiring cells oxidizes the respirable substrates, and the potential energy in these substrates is thereby released and rendered available for vital processes.

It cannot be too strongly emphasized that the fundamental event in respiration, the setting free of potential energy, occurs within and is governed by the protoplasm of living cells, which implies that dead cells (*e.g.*, cork, wood vessels, fibres, etc.) do not respire. We may

[1] The monographs by Kostytschew (*175*), and Stiles and Leach (*295*), deal with this subject, and in chaps. III and VI in Onslow's book (*214*), many problems of the chemistry of plant respiration are defined.

regard the gaseous exchange that accompanies respiration as an external manifestation of the metabolic events that are governed by the respiratory centres in protoplasm. So far no one has succeeded in assigning respiratory activity to particular parts of the protoplasm.

Certain of the respiratory events in aerobes have analogies with processes that occur in the engines of fuel-driven machines. The engines are so constructed that fuel is rapidly consumed at high temperatures in the presence of oxygen. The chemical energy in the fuel is liberated during the combustion, and some of it (the so-called free energy) is harnessed to drive the machine. Arrangements are made for the escape of the waste gases produced. In respiration, protoplasm absorbs oxygen and consumes the fuel (*i.e.*, the respirable substrates) by slow combustion at ordinary temperatures. One of the fundamental endowments of a living cell is that it can harness in a specific manner some of the energy that is liberated (see also p. 3), and use it to promote vital processes. This transfer of energy occurs with a machine-like precision in the colloidal structures of the protoplasm. Non-gaseous waste products of respiratory combustion (*e.g.*, water) accumulate, and gases (*e.g.*, carbon dioxide) escape.

Carbohydrates of the hexose class are the respirable substrates that will chiefly engage our attention in this chapter. The complete oxidation of 1 gram-molecule of a hexose (180 grams) leads to the liberation of 674 Calories of energy (*cf.* p. 223) :—

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674 \text{ Cals.}$$

1 gram of hexose would yield 3·74 Calories. Weight for weight, fatty oils, which sometimes replace carbohydrates as respirable substrates, particularly in seeds, form a richer energy reserve. The calorific values of fatty oils vary slightly with their composition, but we may take 9·5 Calories as the approximate value for 1 gram of any fat. There is good evidence that proteins, a very varied class of compounds, can, on occasions, serve as respirable substrates. In dietetics the calorific value of 1 gram of protein is taken as 5·7 Calories.

As regards the fate and function of the energy liberated during respiration, unsolved outnumber solved problems (for discussion, see Bayliss, *20*, chap. XX, Briggs (*55*), and Wohl and James (*335*)). It is generally accepted that much of the energy released appears as heat energy, which is not conserved but is at once lost by radiation. Occasionally, under natural conditions (*cf.* conditions in Potter's illustrative experiment, p. 266) when growth is rapid and the surface for radiation small, as in the growing spadices of Aroids, the temperature in the plant is greater than that in the environment. As a result metabolism will become locally hastened for a time, and the growth rate increased. Except when this local advantage temporarily accrues, it is not known in what way plants benefit from the heat energy produced by the consumption of valuable food reserves.

We have already argued from general considerations that a fraction of the energy liberated in respiration promotes certain vital processes, but we cannot as yet cite convincing experimental data to support this argument. The development of Doyer's experimental attack on the problem (see Wohl and James, *335*) may provide data for the construction of balance sheets of energy exchanges, which, conceivably, will reveal the desired evidence. Doyer's idea was to measure simultaneously the CO_2 output, heat production (H Calories) and loss in calorific value (C Calories) of the total dry matter of germinating seeds over a certain period and then to calculate the total energy (E Calories) liberated by respiration from the relation that 2·5 Calories of chemical energy become available for every gram of CO_2 produced by a tissue whose respiratory quotient is unity. If from the results of an experiment in which all the technical difficulties of accurate measurement are overcome, it is found that E is the same as both H and C, it could be inferred that all the energy liberated in respiration is transformed into heat. Supposing, however, that both H and C turned out to be less than E, a possible conclusion would be that a fraction (F Calories) of the respiratory energy had become bound in those anabolic products (proteins, lipoids, etc.) which are continuously being synthesized during growth.

We have already on pp. 54 and 69 considered examples of the coupling of anabolic processes and catabolic processes of respiration. The fundamental fact that energy set free in one metabolic process can be used in another is admirably illustrated by the behaviour of certain autotrophic micro-organisms, which obtain energy by the oxidation of inorganic materials (see p. 247). These peculiar oxidations by specialized forms of bacteria are sometimes classified with respiratory processes because oxygen is absorbed and energy liberated. It must be remembered, however, that there is no evidence that this energy is used for any vital process other than the chemosynthesis of carbohydrates, and there is good reason to believe that in these autotrophic organisms the " energy for life " comes not from inorganic materials, but from organic respirable substrates that are produced by chemosynthesis and subsequent metabolism. It will doubtless be possible to give a critical definition of the term respiration when a deeper understanding of this function has been gained. It may well be that the oxidations of inorganic materials by autotrophic bacteria will then be clearly distinguishable from true respiratory processes.

B. Experimental Methods

(i) CO_2 output[1] and oxygen uptake during the aerobic respiration of green higher plants. The manner of expressing the rate of respiration, or, as it is

[1] It has not been unusual in the past to regard the terms CO_2 *output* and CO_2 *production* as having the same meaning except when green plants are respiring

sometimes termed, the respiratory intensity, varies according to the experimental material used and the purpose in view. Thus, the volume or weight of carbon dioxide liberated or of oxygen absorbed is frequently referred to the fresh weight or dry weight of the respiring tissue. For leaves, however, unit surface is sometimes taken as a basis for reference. In long duration experiments on growing plants it must be remembered that changes in weight occur as well as changes in the number of respiring cells (*cf.* pp. 277–9).

To measure the rate of production of carbon dioxide the apparatus sketched in fig. 33 may be used. Air is first freed from carbon dioxide by passing it through a soda-lime tower. It is then passed through lime water, which will remain clear, if the soda-lime has done its work properly. The respiratory carbon dioxide is absorbed from the air issuing from the experimental chamber by a known volume of a stock solution of baryta contained in a Pettenkofer tube (so-called after the experimenter who first used this form of tube). The rate should be slow enough to allow the bubbles to pass singly, without fusing, through these long narrow tubes. The change caused by the formation of barium carbonate is measured by

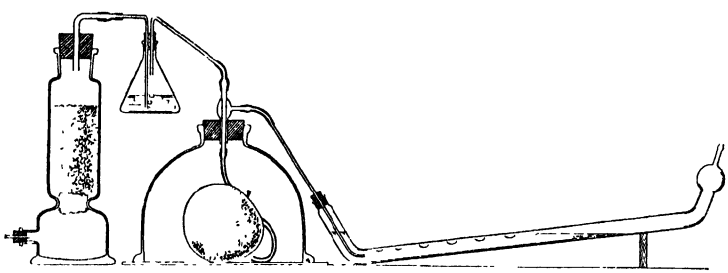


FIG. 33. Apparatus for measuring the rate of CO_2 output in aerobic respiration (see text, p. 261), or in anaerobic respiration (see text, p. 264).

titrating the solution against standard hydrochloric acid, and from the titre the amount of carbon dioxide given off in unit time may be calculated. For further details concerning this continuous-current method see Kostytschew (*175*).

The principle underlying many methods of measuring oxygen uptake is illustrated in experiments with Ganong's respirometer (see Ganong, *95*). Two c.c. of respiring plant material are put in the bulb of the respirometer (fig. 34), and a ten per cent. solution of caustic potash is placed in the manometer. At the outset the ground-glass stopper is turned until the hole bored in the stopper coincides with that in the neck of the container. The air around the material will then be at atmospheric pressure. The

in the light, *i.e.*, when only a fraction of the CO_2 produced by respiring cells is liberated from the plant because some of it is consumed in photosynthesis. Evidence has now been obtained (see p. 329) that CO_2 may serve as a metabolite even in the dark when the substances named in fig. 1 are being produced. Accordingly, when plants are synthesizing vegetable acids or their derivatives, CO_2 output may be less than CO_2 production. In revising this edition, therefore, we have made an effort to distinguish between the terms CO_2 *output* and CO_2 *production*. For instance, in considering the oxidative metabolism of carbohydrates, we argue that output and production may have different values except when it is known that the respiratory quotient is exactly unity.

levelling tube is adjusted so that the potash in the graduated tube is at the 100 c.c. mark. Seeing that the volume of the apparatus up to the 100 c.c. mark is 102 c.c., and that we have introduced 2 c.c. of plant material, the respiring tissue is at the start surrounded by 100 c.c. of air. The experiment is begun by turning the glass stopper to cut off communication with the outside air. Respiration now takes place in a closed space, and the carbon dioxide liberated is absorbed by the potash. The solution rises in the graduated tube until the mark 80 is reached, when the potash is adjusted so as to be at the same level in the graduated and the levelling tubes. It then remains at this level, *i.e.*, one-fifth and only one-fifth of the air is absorbed during aerobic respiration. Plainly this fraction represents oxygen. Hence we may infer that CO_2 output is accompanied by oxygen uptake. The rate of ascent of potash can be taken as a rough measure of the rate of oxygen uptake.

The same apparatus may be used to determine the respiratory quotient, *i.e.*, to compare the volumes of oxygen absorbed and carbon dioxide liberated over a period. In this experiment a saturated solution of common salt is first placed in the manometer. The saline solution should be used, since carbon dioxide dissolves in pure water. It is far less soluble in strong salt solution. The fact that it is slightly soluble will, of course, affect the result.

When carbohydrates are being oxidized, the level of liquid will remain approximately constant, *i.e.*, measured in c.c., the CO_2 output and oxygen uptake are the same and the R.Q. = 1. If pellets of solid caustic alkali are now added to the salt solution the carbon dioxide that has accumulated will be absorbed, and can thus be measured. The number obtained will, of course, also represent the amount of oxygen absorbed.

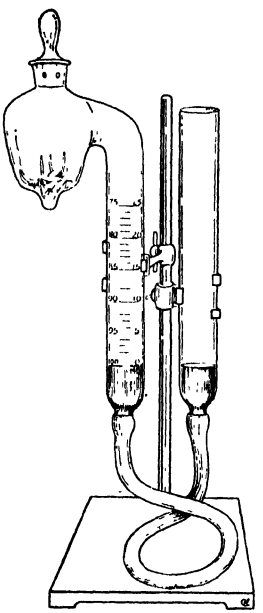


FIG. 34. Ganong's respirometer (see text).

If fats are being oxidized the level of liquid rises. This means that the respiratory quotient is less than unity, *i.e.*, a greater volume of oxygen has been absorbed than carbon dioxide given out. Let us suppose that the excess oxygen is V_1 c.c, and that when the potash pellets are added there is a further reduction of V_2 c.c. The value of the respiratory quotient will then be given by $V_2/(V_1 + V_2)$.

In Ganong's respirometer changes in temperature alter the volume of the gas in the enclosed space around the respiring material. In order to correct for this alteration a second apparatus should be set up in which 2 c.c. of moist nonliving material (*e.g.*, cotton wool) takes the place of the plant material in the respiratory chamber. Such increases or decreases in volume as are attributable to changes in external conditions should respectively be added to or subtracted from the reductions in volume observed in the apparatus containing the plant material.

Several other types of apparatus may be constructed from wide-mouthed bottles, rubber stoppers, and glass tubes, to illustrate the principles underlying the use of precision apparatus such as Barcroft's differential respirometer or Warburg's constant volume respirometer. These respiro-

meters have been widely used in measuring the rate of respiration of isolated animal tissues and bacterial and algal cells, the rate of oxygen uptake shown by non-living systems containing oxidation enzymes (see Dixon, *77*), and more recently (see, for example, Turner (*316*) and Boswell and Whiting (*43*)) the rates of CO_2 output and of oxygen uptake by thin slices of tissues of the higher plants. In the measurement of oxygen uptake in respiration carbon dioxide is absorbed, as in Ganong's apparatus, by ten per cent. caustic potash, which should be placed in a sample tube that stands upright in the wide-mouthed respiratory chamber. The surface for the absorption of carbon dioxide may be increased by dipping rolls of filter paper into the potash. In a differential respirometer a manometer is placed between the respiratory chamber and a second vessel of the same volume. This vessel acts as a compensating chamber, so that the readings on the manometer will not be affected by such changes of temperature or of barometric pressure as may occur during the experiment. For the measurement of the rate of oxygen uptake by non-living systems containing oxidation enzymes, it is not necessary to place potash in the experimental chamber, since carbon dioxide is not usually liberated when such systems are in action.

Finally we note that by gas analysis changes in the concentrations of oxygen and carbon dioxide around plant tissue placed in a closed system may be readily determined. Haldane's apparatus (Haldane, *112*) has been much used in such gas analyses.

(ii.) **CO_2 output and the accumulation[1] of ethyl alcohol and acetaldehyde in anaerobic respiration.** If plant tissue, in which carbohydrates are being oxidized, is placed in Ganong's respirometer, with the level of neutral saline solution in the graduated tube originally at the 80 mark, the level will remain constant for a period, but will begin to fall when all the oxygen has been used in aerobic respiration. Subsequently a gas will be given off without the absorption of any gas from the environment, and the rate of fall of the level of liquid in the closed limb of the manometer may be used as a measure of anaerobic respiration. That this gas is carbon dioxide can be shown by introducing potash pellets, when it will be found that the level of liquid in the closed limb will rise above the 80 mark. The rise to the 80 mark may be attributed to the absorption of the carbon dioxide produced in anaerobic respiration, and the rise above this mark to the absorption of the carbon dioxide liberated during the preliminary phase of aerobic respiration.

The rate of production of carbon dioxide under anaerobic conditions is, however, best found by using the apparatus sketched in fig. 33, but substituting for the air current a current of nitrogen from a gas cylinder.

According to Stiles the production of carbon dioxide under anaerobic conditions was first reported by Cruickshank in the eighteenth century. About 1870, Pasteur demonstrated that fermentation occurs in the higher plants in the absence of oxygen. Many investigations have been made since that date on the production of ethyl alcohol. Traces of other products of zymase cleavage (*e.g.*, acetaldehyde) may also accumulate under anaerobic conditions. Thomas (*303*) developed methods for measuring the concentrations of ethyl alcohol and acetaldehyde in plant tissues; and,

[1] Accumulation is, of course, evidence of production, but a substance might be produced without accumulating were it completely consumed immediately it was produced (see p. 214). Existing evidence (see p. 305) suggests that we may infer that ethyl alcohol is not produced by green plants, unless it accumulates. It is an end-product of metabolism. Acetaldehyde, however, may possibly be produced on occasions without accumulating. It may be an intermediate product of metabolism.

using these methods, he obtained data from which progress curves were drawn recording the accumulation of these substances in apples placed in pure nitrogen (fig. 39). At the time these experiments were carried out the term *fermentation* suggested to many people that products were being formed by the metabolic activity of micro-organisms. Accordingly, Thomas described by the term *zymasis* the process which causes ethyl alcohol and other products of zymase cleavage to accumulate in plant cells. He described the process as *anaerobic zymasis* when it occurred in the absence of oxygen. Other forms of zymasis have since been studied (p. 303).

The occurrence of zymasis is easily demonstrated when acetaldehyde is among the products that accumulate. Acetaldehyde is a volatile substance and escapes from the tissue. The volatilized aldehyde may be absorbed by a solution containing one per cent. phloroglucinol dissolved in six per cent. sulphuric acid. Under these conditions it combines with phloroglucinol to yield aldehyde phloroglucid, which is only slightly soluble in dilute acid. During the course of certain forms of zymasis (*e.g.*, CO_2 *zymasis*, *HCN zymasis*, H_2S *zymasis*) in apples and many other tissues, sufficient acetaldehyde accumulates to cause the appearance after a day or two of a copious precipitate in the phloroglucinol reagent when the gas issuing from the respiratory chamber is led through this reagent (see Thomas, *loc. cit.*). Moreover, at the end of an experiment on a bulky organ, such as an apple, a blue colour may be obtained when first a solution of sodium nitroprusside and then a solution of piperidine are applied to a cut surface of flesh tissue. This is known as Rimini's specific test for acetaldehyde. It may also be used to detect acetaldehyde in steam distillates of tissues that have undergone zymasis. In control experiments with apples kept in pure air, little or no phloroglucid will be precipitated, and at the end of the experiments the flesh tissue will not give a blue colour when subjected to Rimini's test.

(iii.) **Organic compounds produced during cellular oxidations.** The production of vegetable acids is discussed in chap, XIV.

(iv.) **Loss of dry-weight and disappearance of respirable substrates.** Quantitative experimental methods (p. 224) permit changes in the carbohydrate content of respiring tissues to be determined. Thus it has been shown that the total carbohydrate decreases during the germination of starchy seeds (p. 207). The consumption of carbohydrates also accounts for the disappearance of starch from the cells of green leaves respiring in the dark. More specialized books (*e.g.*, Haas and Hill, *108*) must be consulted for the description of methods for measuring changes in the fat content and protein content during respiration.

CO_2 output leads to a loss of carbon, and consequently to a decrease in dry weight. Decreases in the content of carbon, hydrogen, and oxygen have been determined by the combustion of comparable samples of germinating seeds dried before and after a period of respiration. Changes in dry weight during the germination of seeds are readily determined (see table, p. 206), and the loss in dry weight when green leaves are respiring in the dark may be demonstrated by the half-leaf method (p. 222). It should be noted that the dry weight of all green plants tends to decrease at night. Deciduous perennials, also, lose dry weight in the winter and during the early stages of growth in the spring. Thus when growth is revived in bulbs, corms, tubers and rhizomes, and when the buds on the twigs of woody perennials swell and begin to develop into leafy shoots, respirable material in storage parenchyma is drawn on and oxidized. Consequently the dry weight decreases, and the wastage cannot be repaired until the green leaves unfold and display photosynthetic activity.

(v.) **The liberation of heat energy during respiration.** It was realized over a century ago that the production of what was then called vital heat by certain actively growing parts of plants (*e.g.*, the spadices of Aroids) depended, in some way, upon the absorption of oxygen. That growth is accompanied by the liberation of heat energy was neatly demonstrated by Potter. He placed germinating seeds in thermos flasks and observed that the temperature rose continuously during the first few days (see fig. 35). In the flask containing seeds that had previously been killed by immersion in boiling water, the temperature did not rise until micro-organisms began

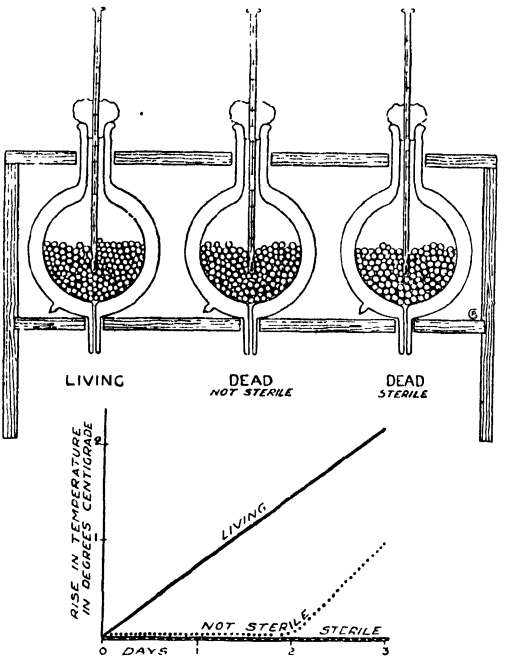


FIG. 35. Potter's method of demonstrating the liberation of heat which accompanies the germination of seeds, and the growth of micro-organisms (see text). The results of an experiment are graphically represented in the lower figure.

to multiply rapidly. This happened after two days. In the flask containing the seeds that had been killed by immersion in boiling one per cent. mercuric chloride (a poison to all forms of protoplasm) the temperature remained constant throughout the experiment. The mercuric chloride kept the killed seeds free from micro-organisms. It was thus demonstrated that heat energy was liberated only when the embryos of the seeds were growing, or when micro-organisms were multiplying. It may be presumed that the energy was liberated in a respiratory process.

C. Respiratory Quotients

The simultaneous measurement of CO_2 output and oxygen uptake by a plant tissue permits the calculation of the respiratory quotient

(R.Q.), *i.e.*, the number expressing the ratio of the volume of carbon dioxide given out/volume of oxygen simultaneously absorbed by a given mass of tissue over a given period. The value of this ratio is governed in the first place by the chemical nature of the substance that is oxidized. Thus, for example, a comparison of the equations that represent the complete oxidation of (*a*) carbohydrates, and (*b*) fats, shows that for the former this quotient would be unity, and for the latter 0·7 approximately.

(*a*) $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$.
(*b*) $2C_{51}H_{98}O_6 + 145O_2 = 102CO_2 + 98H_2O$.

The respiratory quotients for different fats vary slightly. They are governed by the molecular weights of the constituent fatty acids. For the complete oxidation of organic acids the R.Q. would be greater than unity : for example, the R.Q. for oxalic acid would be 4, that for tartaric acid 1·6. Its value for proteins fluctuates about 0·5.

Some diversity exists in the nature of the metabolic events in which oxygen is absorbed and carbon dioxide produced ; and it is not yet possible to give a completely satisfactory definition of respiration in terms of gaseous exchange. Thus oxygen is always absorbed when tissues containing direct oxidases change colour after injury, when anthocyanins are being formed in healthy plants, and when fats are being converted to carbohydrates. And in passing from a hexose unit to a pentose unit, a degradation that probably occurs in many developing plant cells, oxygen uptake may accompany the change of the hexose to the corresponding uronic acid, and carbon dioxide is produced by the decarboxylation of this acid. Consequently when these and other such changes are occurring in respiring tissues, it is not easy to interpret the meaning of numbers that express respiratory quotients. Nevertheless experience has shown that when it is known what types of respirable substrates are present in cells, these numbers often point decidedly to the substrate that is actually being oxidatively consumed, and to the mode of consumption. Thus, considering the data given in table XII, we may infer that carbohydrates were completely oxidized to carbon dioxide and water when the respiratory quotients were near to unity for tissues known to contain carbohydrates (*e.g.*, many green leaves, germinating starchy seeds including wheat seedlings respiring in more than five per cent. oxygen, and healthy green apples from storage chambers).

The respiratory quotients recorded in the table worked out at less than unity, (*a*) when some substrate other than carbohydrate was consumed, (*b*) when carbohydrates or other substrates were incompletely oxidized, and (*c*) when oxygen uptake occurred in processes other than respiration. Thus in germinating linseed, the principal food reserve is fatty oil, and the R.Q. of 0·64 accords well with the

approximate value 0·7 for the complete oxidation of a fatty oil. The low value of the apparent R.Q. for apples that turned brown in air containing chloroform vapour may be attributed to the fact that changes of colour following injury are accompanied by oxygen uptake that has no concern with respiration. The brown substances formed are oxidation products of polyphenols.

TABLE XII. *Respiratory Quotients*

	R.Q.
Many leaves rich in carbohydrates	1
Darkened shoots of Opuntia	0·03
Germinating starchy seeds	1
Healthy green apples in air stores	1
Wheat seedlings in 5–20 per cent. oxygen	0·93–0·98
,, ,, 3 per cent. oxygen	3·34
,, ,, nitrogen	infinity
Germinating linseed	0·64
Maturing linseed (apparent R.Q.)	1·22
Germinating buckwheat seeds (first five hours)	0·47–0·50
Germinating peas with testas on (first seven days)	2·8–4
Germinating peas with testas off	1·5–2·4
Old apples in air stores	>1·3
Apples browning in air containing chloroform vapour (apparent R.Q.)	sometimes<0·25
Apples treated with HCN or H_2S	sometimes>2

In the light of the arguments set forth on pp. 327-9 a possible meaning of the low R.Q. for Opuntia respiring in the dark may be suggested. Conceivably the normal respiratory oxidation of hexose and the synthesis of dicarboxylic acids and their derivatives are occurring as collateral processes. Some, or even all of the CO_2 *produced* in the reaction represented above by the overall equation (*a*) may be consumed as a metabolite in the production of acids according to the overall equation

$$C_6H_{12}O_6 + 2CO_2 = 2C_4H_6O_5.$$

If all were consumed the CO_2 *output* by the whole shoot would be zero, and, since the oxygen uptake is positive, the R.Q. would be zero. In the experiment which gave the result recorded in Table XII, a small fraction of the respiratory CO_2 escaped and, accordingly, a positive but very low R.Q. was obtained. In the development of the argument elsewhere, but not in this book, evidence from literature dating back to the days of de Saussure will be marshalled to show that apparent R.Q.'s ranging from zero to nearly unity have been recorded for tissues of succulent and other plants in which acids accumulate in the dark, or in some instances (*e.g.*, fleshy fruits) even in the light. Evidently, according to the present writer's interpretation, the value of the apparent R.Q. will approach closer to unity as the rate of CO_2 production in real respiration increases relatively to the rate of CO_2 consumption in the synthesis of acid. In 1946 Beevers [1] obtained evidence at Newcastle supporting this view. He exposed darkened leaves of *Bryophyllum sp.* to gas mixtures containing nitrogen, oxygen and low concentrations of carbon dioxide. At first acid accumulated in

[1] Unpublished work, but the results were reported at a meeting of the Society of Experimental Biology, held in London in January, 1947.

the leaves, and while this was happening CO_2 as well as oxygen was absorbed by the leaves, *i.e.*, a negative apparent R.Q. was observed for darkened green shoots, possibly for the first time. There was little doubt that the CO_2 absorption related to the metabolism leading to acid production, since it had ceased when the curve for acid accumulation reached its peak. Subsequently CO_2 was liberated by the leaves, and at a rate approximately equal to that of oxygen absorption, *i.e.*, during this phase the measured R.Q. was unity. Since the oxygen uptake had continued at a steady rate from the beginning of the experiment, it is inferred that the respiratory oxidation of hexose was in progress throughout, but that, while acid was accumulating, the CO_2 produced was, in addition to the CO_2 absorbed from the environment, consumed in the synthesis of acid. We have not yet sufficient data to record the gradual change from a negative apparent R.Q. to a real R.Q. of unity, through apparent values ranging from zero up to, but not including, unity.

The fact that when anaerobic respiration occurs (*e.g.*, when wheat seedlings respire in the absence of oxygen) the R.Q. must be represented by the infinity sign permits us to interpret some of those R.Q.s which work out at greater values than unity for tissues respiring in gas mixtures containing oxygen. Thus for the wheat seedlings respiring in three per cent. oxygen or less, it may be suggested that respiration was partly aerobic (characterized by an R.Q. of unity) and partially anaerobic. For peas germinating in air with their testas on (R.Q. 2·8–4·2) or with their testas off (R.Q. 1·5–2·4) we may again infer that carbohydrate catabolism was partially anaerobic. This may have been owing to the restrictions imposed by the testas and the bulky cotyledons to the supply of oxygen to the respiring cells. Apparently the removal of testas improved aeration. The R.Q.'s greater than 1·3 indicate that anaerobic respiration had occurred in old apples respiring in air, possibly owing to incipient cell disorganization. As a result of this anaerobic respiration old apples in air stores and germinating peas may contain ethyl alcohol, which, as we shall see later, is only produced by higher plants when, through restricted gaseous exchange or some other cause, oxidative activities are diminished. It will be noticed that apples treated with hydrogen cyanide or hydrogen sulphide gave high respiratory quotients. Under these conditions also alcohol is produced.

It is probable that in the experiment with maturing linseed, carbohydrates were simultaneously being oxidized (R.Q. of unity) into carbon dioxide and water, and being changed into fatty oils. This latter change reduces the relative amount of oxygen in organic combination, and presumably in the maturing linseed this released oxygen took part, either in the elementary form or in combination in the oxidation of the carbohydrates, and consequently reduced the amount of oxygen absorbed. We can thus understand why the apparent R.Q. (1–2) was found to be greater than unity.

Stiles and Leach (*294*) attributed the low R.Q. in the first few hours

after buckwheat seeds have imbibed water to the conversion of the small reserves of fat in the seed into carbohydrate. It should be noted that the apparent R.Q. for the conversion of fat into carbohydrates is less than the true R.Q. for the respiratory oxidation of fat. Using a wide range of plants, Stiles and Leach have made determinations of the changes in the R.Q. during the early stages in the germination of seeds which contain different food reserves (*e.g.*, starch, hemicellulose, and fatty oil). Thus an R.Q. of unity for yellow lupin seeds was related to the oxidation of a hemicellulose built up of arabinose and galactose, as well as to that of glucose and fructose. By using a delicate instrument called the katharometer for the measurement of oxygen uptake and CO_2 output they were able to determine the respiration of single seeds. They found that in each species examined the quotient changed during germination. They attributed these changes to the fact that different food reserves were used at different stages in development. Respiratory quotients near to unity were related to the oxidation of carbohydrates, and those near to 0·75 to the oxidation of fats. Thus with maize grains, which contained a little sugar, much starch, and some fat, they found that the quotient fell from an initial value of unity to 0·75, and later rose to unity once more. They suggested that at the outset sugar acted as the respirable substrate, that fat was oxidized later, and that later still starch was consumed. With buckwheat seeds they found that after the first few hours the R.Q. rose steadily from 0·5 to unity, and concluded (*a*) that the reserve of fat was quickly consumed, and (*b*) that carbohydrate subsequently acted as the respirable substrate.

D. Respiratory Substrates

Although the value of a respiratory quotient may suggest what class of substance is being oxidized and whether oxidation is partial or complete, clear evidence can only be obtained by measuring simultaneously the consumption of possible respiratory substrates and the production of possible end products. Such measurements were made in the last century. For example, the results discussed on pp. 206 and 269 showed that in germinating seeds carbohydrates disappeared when the respiratory quotient was unity, and that fatty oils were consumed when the respiratory quotient was 0·7. The results of quantitative analyses considered with values obtained for respiratory quotients indicated that, excepting in germinating oily seeds, carbohydrates usually supply the respirable material consumed by most of the tissues of Angiosperms. None of these earlier experiments provided any evidence of the oxidation of nitrogenous organic compounds. A widely held view that the proteins of protoplasm undergo respiratory oxidation and that carbohydrates and fatty oils serve for the regeneration of proteins had been discredited by the

beginning of the present century. The fact should be noted, however, that certain fungi and bacteria can be grown in media completely free from sugars. For example, it has been proved that peptones may act as a source of food for the growth of mould fungi and at the same time be oxidized by these organisms.

Respiratory substrates in green leaves. In normal life green plants are not likely to suffer from extreme carbohydrate starvation, but such a state may be experimentally induced by keeping them for prolonged periods in the dark. F. F. Blackman measured the CO_2 output of detached cherry laurel leaves whilst they gradually starved in the dark. After a period of steady respiration the rate fell to a value that was about a quarter of the original steady value. No measurements were made of changes in the concentration of possible respiratory substrates, but it was reasonably assumed that sources of carbohydrate (sugars, polysaccharides, glycosides) were at first tapped and that what Blackman termed *floating respiration* gradually declined in rate as the supply of these subtrates diminished. The residual respiration, which Blackman termed *protoplasmic respiration*, remained more or less constant for a period. Implicit in this term is the suggestion that proteins and other substances in the protoplasm may be oxidized when shortage of carbohydrate becomes acute. The possibility could not be excluded that protoplasmic respiration proceeds in addition to floating respiration under normal conditions even in attached leaves and even when cells are provided with an ample supply of carbohydrate. Were evidence supporting this possibility to be obtained, the discredited view concerning the part played by proteins in respiration would have to be considered anew. Evidently Blackman's experimental results defined many problems for future biochemical investigation. Finally we note that after a period of protoplasmic respiration cells are doomed eventually to autolyse and die. Leaves kept in the dark gradually become yellow and then brown. Blackman found that during autolysis the rate of respiration of cherry laurel leaves changed. For a short period there was an increase above the previous steady rate of protoplasmic respiration ; but after a maximum had been reached the rate gradually fell and became zero at the death of the leaf.

Deleano (*73*) formulated the exacting requirements for establishing the fact that carbohydrates alone are undergoing oxidation. He pointed out the necessity of measuring changes in the concentration of carbohydrates, proteins, fats, organic acids and other possible respiratory subtrates, during a period of respiration in which oxygen uptake and CO_2 output are also determined. Then he maintained, it would have to be shown that of these substrates only the carbohydrates are oxidatively consumed, and that the amount of total

carbohydrate lost corresponds to the amount of the oxygen absorbed and of the carbon dioxide emitted. Deleano made experiments on the respiration in the dark of detached leaves of *Vitis vinifera*. From his results he concluded that, during a period of one hundred hours, all the carbon dioxide produced came from carbohydrates and ultimately from the starch reserve in the blades. He did not detect any change in the concentration of coagulable protein or of soluble nitrogenous material in the cell sap until all the starch had disappeared. Not until the leaves had been starving in the dark for over four days did proteins give rise to soluble organic products and ammonium salts. Deleano inferred that although leaves possess the power of oxidizing proteins, these and other nitrogenous substances are actually consumed only under conditions of extreme want, such as are not likely to be experienced by leaves attached to plants growing in the field. He maintained that under natural conditions only carbohydrates are oxidized.

The experiments of Yemm (*337*), which may be regarded as being in direct succession to those of Blackman and Deleano, have an excellence of their own in that measurements were made by modern methods of changes not only in the concentration of total carbohydrate, but also in that of various carbohydrate fractions. Leaves of a selected variety of barley were picked in the late afternoon, *i.e.*, when, as a result of photosynthesis, they would be well stocked with carbohydrates. It should be noted that barley leaves may contain starch (but see p. 198) and a fructosan polysaccharide in addition to cane sugar, glucose, and fructose. Comparable samples of leaves were placed in the dark; one set of samples was used for the determination of the drift of CO_2 output with starvation time, and another set for the measurement of the simultaneous consumption of carbohydrates. The results of one of the experiments are graphically represented in fig. 36. We shall ignore the preliminary rise in CO_2 output, as it may possibly have resulted from the handling of the leaves in setting up the experiment (see p. 281). The first facts to note are that after twelve hours the respiration began to fall rapidly and that this fall continued until about the fortieth hour, when it was arrested. Subsequently the leaves turned yellow, and the respiration became variable. In other experiments Yemm found that this yellowing phase continued until about the 140th hour, after which time the rate of CO_2 output progressively declined and the leaves turned brown as the cells died. Broadly, the drift during starvation was similar in barley leaves to that observed for cherry laurel leaves by F. F. Blackman. It should be noted, however, that barley leaves died after about five or six days, whereas cherry laurel leaves did not turn brown until they had spent about twenty days in a darkened chamber.

The results charted in fig. 36 make it clear that there was a continuous respiratory consumption of carbohydrates in the darkened leaves. After a hundred hours, carbohydrate substrates had been exhausted excepting for small amounts of sucrose and glucose. Yemm pointed out that no simple quantitative relationship appears to exist between the concentration of any individual carbohydrate and the rate of CO_2 production. None of the major variations in this rate can be correlated directly with concurrent changes in the concentration of one particular sugar. Sucrose was the chief source of hexose present in the leaves at the outset, and the fall in the concentration of this sugar during the first forty hours is striking. It is important to note that free fructose completely disappeared during the first twenty hours or so in spite of the continuous production of this sugar by the hydrolysis of sucrose and the hydrolysis of fructosan. This clearly suggests that fructose is a sugar that is very readily oxidized. The amount of starch present gradually diminished. If starch was hydrolyzed with the production of glucose it is not surprising that this hexose accumulated for a period, especially as it was also being formed by the hydrolysis of sucrose. But glucose must also have been consumed during this period, since the maximum concentration found was less than that which would have been reached had starch and sucrose been hydrolyzed, and had no such consumption of the resulting glucose occurred. The steady consumption of glucose after about forty hours was proved beyond doubt by the results recorded in fig. 36. The inference that glucose is an oxidizable carbohydrate appears to be justified. It remains possible that it is less easily oxidized than fructose.

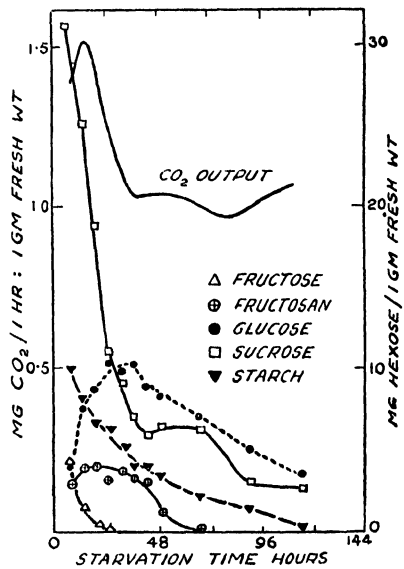


FIG. 36. Changes in the rate of CO_2 output and in the concentration of carbohydrates in respiring barley leaves starving in the dark (from Yemm, see text).

It will be recalled that Deleano emphasized the importance of relating the rate of CO_2 output to that of the exhaustion of total carbohydrate, and of paying regard to the rate of oxygen uptake.

After analysing his results Yemm drew similar conclusions to those of Deleano. He states that only in the first stages of starvation can the carbon dioxide produced be said " to arise solely from the normal sources of hexose estimated by analysis." He found that the respiratory quotient was about unity during this period. With barley leaves this first period was of short duration, lasting for less than twenty-four hours, *i.e.*, for a much shorter period than the hundred hours of exclusively carbohydrate respiration found for vine leaves by Deleano. It should be noted, however, that twenty-four hours is much longer than the maximum starvation period through which barley leaves have to pass in the field during the nights of a growing period.

Yemm found that progressively with starvation time there was an increasing component of the CO_2 output which did not arise from carbohydrate. Indeed, throughout the yellowing phase, *i.e.*, from the fortieth to the 140th hour, less than twenty-five per cent. of the total CO_2 output could be accounted for by carbohydrate loss. He also observed that during the period between the twentieth and the fortieth hour the respiratory quotient fell from unity to 0·8, and was maintained at this lower value whilst the leaves turned yellow. He pointed out that such a lowering of the respiratory quotient would occur if proteins or their derivatives replaced carbohydrates as the substrates used in respiration. The evidence obtained by Deleano of the consumption of proteins by vine leaves after starving for four days in the dark, and the suggestion based on Blackman's experiment that starved cherry laurel leaves may consume proteins, will be recalled.

In later experiments Yemm (*338*) made quantitative investigations on the protein catabolism of detached barley leaves starving in the dark. His results indicated that protein nitrogen is progressively hydrolyzed with the production of amino acids and amides, and that during the yellowing phase the concentrations of some of these fractions decrease, strongly suggesting that they are then used in respiratory oxidations. An interesting point was that free ammonia accumulated during the late phase of yellowing. Yemm suggested that this toxic substance may have hastened the death of the leaves.

Having obtained evidence that plant protoplasm is capable of oxidatively consuming products arising from the hydrolysis of protein, Yemm discussed possible methods by which these products might give rise to nitrogen-free substances that could undergo decarboxylation, *i.e.*, could produce carbon dioxide. Clearly great interest attaches to work at present in progress on the activities of enzymes that oxidize amino compounds and amides, or their nitrogen-free derivatives. To what extent the activities of such enzymes are a normal feature of respiratory processes in leaves and other organs is a

matter for further investigation. At the conclusion of his work on barley leaves Yemm expressed the view that " it is highly probable that under normal circumstances the respiration of higher plants is dominated by carbohydrate changes and that proteins are relatively less important with the possible exception of some germinating seeds."

Respiratory substrates in the potato tuber. Since starvation effects are not soon met with in underground storage organs containing abundant starch, such organs afford suitable material for prolonged investigations on the respiratory oxidation of carbohydrates. For example, in certain experiments on the potato tuber, lasting for two months or more, the value of the respiratory quotient remained at unity. It has long been known that the equilibrium between starch and sugar in cells is disturbed in favour of hydrolysis to sugar by lowering the temperature. Since this fact was discovered the potato has been used in several important investigations on the factors that control the rate of respiration. Outstanding interest attaches to the results obtained by Barker (*14*). In his earlier experiments he altered the concentration of extractable sugar by varying the temperature of storage over the range − 1° C. to + 15° C.; and he measured the respiration. He plotted his results as curves representing the relation between the rate of respiration and the concentration of total sugars. These are described as R/S curves. They approximated to the form of a rectangular hyperbola, such as is obtained when rates of enzyme action are plotted against substrate concentration. Accordingly Barker suggested that the concentration of total sugar in some way determined the concentration of respiratory substrate. His later investigations were more detailed, and were carried out over the temperature range + 1° C. to + 10° C. The results were plotted as before, and Barker attached importance to the fact that not only the R/total sugar curves, but also the R/sucrose curves, approximated to the form of a rectangular hyperbola, but that neither the R/fructose nor the R/glucose curves were of that form. He states that " So far as we are aware the data here presented constitute the first published evidence of a broadly enzymatic relation between the respiration of a plant tissue and the concentration of a particular internal constituent sugar." Barker saw in his results further evidence that sucrose is the principal substrate used by the potato tuber in its respiration. As would be expected, since the value of the temperature coefficient for respiration is usually greater than two (p. 282), the rate of respiration at first fell to a lower value when the tubers were transferred from 10° C. to 1° C.; but a central feature of Barker's work is that he found that, instead of remaining steady at this lower value, the rate of respiration gradually rose to a higher steady value. During the first few days of this rise the sucrose concentration increased without

a corresponding increase in the concentration of hexoses. This meant a rise in the sucrose/hexose ratio, which Barker evaluated from the results of most of his experiments. When the potatoes were returned to a room kept at 10° C. the respiration rate at first increased rapidly; but instead of remaining steady at a higher value characteristic of the higher temperature, a decrease set in after one day. Barker attributed this decrease to a fall in the sucrose concentration that took place at about the same time. Presumably sugar was being reconverted into starch at the higher temperature. On the other hand, the concentrations of glucose and fructose increased for several days, and did not show unmistakable signs of falling until five days had passed, *i.e.*, the CO_2 output decreased while the hexose concentration was rising.

The part played by fructofuranose in respiratory oxidation. The evidence concerning the respiratory consumption of carbohydrates that has just been presented for barley leaves in the dark and for potato tubers, and that which is reported elsewhere for barley seedlings (p. 207) and apples (p. 210), has led to the general acceptance of the view that glucopyranose, fructopyranose, and all compounds which may give rise to these hexoses (*e.g.*, starch, glucosides, fructosans, the mannans of hemicelluloses, sucrose) are potential respiratory substrates. (We note that the status as respirable carbohydrates of galactans and galactosides, and of pentosans and pentosides, requires further elucidation.) This and other evidence (see, *e.g.*, Onslow, *214*) also indicates that fructopyranose often disappears more rapidly than glucopyranose, and that there is a still more rapid consumption of such fructofuranose as may occur as an "active wing" (Barker's term) of sucrose or raffinose, or as a building stone of a fructosan.

If it is accepted that phosphorylative glycolysis is a preparative phase in the respiratory consumption of carbohydrates (p. 300), a reasonable interpretation of the evidence referred to above is that any substance that can give rise to fructofuranose diphosphate may act as a substrate for respiratory oxidation. The free hexoses that can undergo zymase cleavage are *d*-glucose, *d*-fructose, and *d*-mannose (p. 50), and there exists evidence that these sugars are interconvertible (pp. 218, 430). (We lack evidence concerning enzyme action on galactose and pentoses.) The picture is, therefore, that any of these three hexopyranoses can give rise to fructofuranose diphosphate. The energy for the activation, *i.e.*, for the conversion of a pyranose into a furanose sugar, presumably comes from the active phosphate bonds in ATP (p. 53). Clearly, it may well be that fructose is consumed more rapidly than glucose in producing fructofuranose diphosphate, and that fructofuranose set free from active wings of sucrose molecules, or from other saccharides, is used still more rapidly in the same way.

We cannot yet rule out the possibility of a respiratory oxidation of carbohydrates without preparative phosphorylative glycolysis. Concede such oxidation, then certain chemical evidence has significance in accounting for one possible kind of preferential consumption of fructose and especially of fructofuranose. It has long been known that *in vitro* fructose is more readily oxidized than glucose; and, soon after the discovery of fructofuranose, biochemists laid much stress on the fact that this active fructose is more readily oxidized *in vitro* than is normal fructose.

A final fact to note is that fructofuranose does not accumulate in plant cells. If, when set free from sucrose or some other compound, it is not immediately consumed in some way, it is converted into normal fructose, which accumulates.

E. The Rate of Respiration

A number purporting to represent the intensity of respiration (*i.e.*, the rate of respiration per unit fresh weight, dry weight, or surface) has little meaning unless the age and state of the respiring tissue and the prevailing external conditions are described. If we read a statement that 100 gm. fresh weight of a certain Bramley's Seedling apple in air at 22° C. at a certain date evolved 10 mg. (which would occupy about 5 c.c. at normal temperature and pressure) carbon dioxide per hour, we should remember that a different respiratory intensity might have been shown by the same apple at a different temperature or in a gas mixture containing either less oxygen or more carbon dioxide. Furthermore under fixed external conditions the same apple might show different respiratory intensities at different stages of development on the tree or of storage after harvesting. A few general statements may, nevertheless, be made. First it may be said that under similar external conditions in the spring or early summer respiratory intensity expressed in terms of rate per unit mass tends to a maximum in the actively growing parts of a plant; for example, it is relatively great during the germination of seeds and the sprouting of vegetative or flowering buds. Under such natural conditions 100 gm. fresh weight of actively respiring organs (*e.g.*, germinating seeds) may give off in one hour as much as 30 c.c. of carbon dioxide. Were the respiratory quotient unity, this would mean that 30 c.c. of oxygen would be simultaneously absorbed. Even at 22° C. the Bramley's Seedling apple mentioned above was much less active than are germinating seeds, and there are many living plant tissues which are less active than the flesh tissue of apples.

In the present section we shall consider the separate internal factors, viz., number of living cells, the intrinsic respiratory activity of cells, the water content of cells, and the concentration of respiratory substrates, and the separate external factors, temperature and oxygen concentration, which may influence the rate of respiration. These are the factors which under natural conditions determine the rate of supply of respirable substrates and oxygen to the oxidizing surfaces in protoplasm, and determine the rate at which such substrates are oxidized at these surfaces with the production of carbon dioxide.

Number of respiring cells. It hardly requires an experiment to demonstrate that the rate depends upon the number of living cells. Obviously two apples will give off more carbon dioxide in unit time when together than when experimented upon singly. Similarly during germination, or other form of active growth, the rate of respiration

by the *whole* plant will increase, for the new cells arising in meristematic regions respire actively. Their activity may decrease as they age, but as long as a cell contains protoplasm it will give off carbon dioxide. Respiration ceases in cells (*e.g.*, sclerenchyma) that die during differentiation.

At the end of a growing period, *i.e.*, when there is no further increase in the number of cells, the respiration of a whole plant or whole organ will be governed by the intrinsic activities of the constituent cells and by the concentrations of respirable substrates. The magnitudes of these factors may fluctuate, but eventually the rate of respiration of the whole unit will decline as the respiring power of the protoplasm in the ageing cells decreases and as the stores of respirable substrates are depleted. Briggs, Kidd, and West (*56*), measured the respiration at 10° C. of *Helianthus annuus* during the germination, growth, and flowering of this plant. The respiration of a single plant, measured in mg. carbon dioxide per hour, rose slowly from a value of about 0·06 for the first four days to about 0·3 on the thirteenth day. Then as the plant grew the respiration increased to the value 213 by the 112th day; but it fell to 168 by the 136th day. We note in passing that a fully-grown sunflower may in one day at 10° C. produce by its respiration over 2·5 litres of carbon dioxide and, since the respiratory quotient is unity, absorb over 2·5 litres of oxygen, *i.e.*, the oxygen contained in over 12 litres of air.

The numerical value of the respiration of a whole plant or whole organ must be carefully distinguished from that of the intensity of respiration of the unit involved. Briggs, Kidd, and West expressed the intensity of respiration of *Helianthus annuus* as milligrammes carbon dioxide per gramme dry weight per hour. Their results indicated that during the germination of the seeds and for the first three weeks the respiratory intensity remained fairly steady at about the value 3. Subsequently it declined gradually, reaching a value 0·26 on the 112th day; afterwards during flowering it increased, attaining the value 0·39 by the 136th day. The point we emphasize here is that the respiratory intensity fell during growth in spite of the progressive increase in the number of cells. This was probably because the average daily rate of production of dry matter, mainly by the process of photosynthesis, greatly exceeded the average daily rate of respiration. Some of the products of photosynthesis would have been converted into the non-plastic materials that accumulate in cell walls, *i.e.*, the dry weight increase was rendered permanent, and some into substances (*e.g.*, storage products), which might be consumed later. The fall in dry weight from 818 gm. to 419 gm. between the 112th and the 136th day provided evidence of the consumption of stored respirable substrates. This decrease in dry weight was relatively greater than the simultaneous decrease in the

total respiration of the whole plant; so, in consequence, the respiratory intensity rose.

It is easy to demonstrate that during the early stages of the germination of seeds the respiratory intensity increases. There are two reasons for this increase. The respiration of a seed shows a rising tendency for a period, and the dry weight decreases during germination until green leaves have developed. Fluctuations in respiratory intensity during the later stages of transition from the seed to the green seedling stage require further elucidation.

The intrinsic respiratory activity of a cell. It is reasonable to assume that the *extent and activity of oxidative catalytic surface* in the protoplasm will be one of the prime factors determining respiratory rate. The problem of the enzymic constitution of such surfaces has engaged much attention (p. 313). If, as seems nearly certain, more than one enzyme is concerned with respiratory oxidations the rates of oxygen uptake and of CO_2 production will be determined by the activity of the least active of the enzymes concerned with these processes. It is clear that the differential inhibition or stimulation of any of these enzymes as a result of the ageing of plant cells or of abnormal environmental conditions (*e.g.*, a reduced oxygen concentration, or the presence of relatively high concentrations of carbon dioxide, or of narcotic or poisonous substances) may have marked effects on the rates of oxygen uptake or CO_2 production.

An important point to recall (see pp. 175–6) is that mineral salts dissolved in cell sap may affect the intrinsic respiratory activity of cells not only because they provide elements, such as nitrogen, which are used in the synthesis of respiratory enzymes, but also because phosphate and possibly other ions in these salts may participate in respiratory metabolism. James and Arney have contrived to arrange conditions in which the rate of respiration of barley was determined by the amount of phosphate supplied from outside. In a paper entitled " The Relation of Respiration Rate to the Carbohydrate and Nitrogen Metabolism of the Barley Leaf, as determined by Phosphorus and Potassium Supply," Richards (*245*) has discussed complex problems concerning possible direct and indirect relations between mineral supply and respiration. Special interest will attach in the future to the development of his hypothesis, based on his experimental results, that the respiratory activity of cells may be affected by some aspect of their nitrogen metabolism.

F. F. Blackman has called attention to the possible existence of another factor that may affect the intrinsic respiratory activity of a given cell in which the water content and concentration of substances that can give rise to respiratory substrates are in excess of requirements. He pictured that metabolic reactions may be hindered as a result of the spatial separation of the reactants by impermeable

protoplasmic membranes, and coined the term *organization resistance* to express this "important aspect of protoplasmic control of metabolic rate" (see Blackman and Parija, *32*). The implication is that in living cells metabolic events (such as hydrolysis of carbohydrate reserves), and diffusion, are so organized that the rate of supply of respirable substrate to the catalytic surfaces is under the control of the protoplasm. Thus it is supposed that slow hydrolyses or low permeability of membranes would cause respiration to proceed at a slow rate. On the other hand, if the organization resistance decreased a given amount of respiratory substrate would be more rapidly consumed.

The pioneer work of Blackman and Parija on the respiration of apples has been developed by other workers, and the concept of organization resistance has proved useful in the interpretation of the results that they have obtained (p. 211). Huelin and Barker (*144*) have recently attributed to changes in organization resistance some of the complicated effects induced by treating with ethylene sweetened and unsweetened potatoes (see p. 212). Broadly the stimulation of respiration at 15° C. was more marked in potato tissue that had been stored at 15° C. than in tissue that had been stored at 1° C., *i.e.*, than in tissue in which the concentration of sugars was relatively high. The suggestion was made that ethylene brings about a decrease in the organization resistance of the cells, and consequently promotes the supply of sugars to the respiratory centres of the protoplasm. No evidence was obtained of the stimulation or inhibition of respiratory enzymes by ethylene. It was inferred that in potatoes stored at 15° C. the sugar concentration was so low that the rate of supply of respiratory substrates rather than the activity of respiratory enzymes determined the rate of respiration. If treatment with ethylene enhanced the supply of substrate, the rate of respiration would be augmented. On the other hand, the view was put forward that in potatoes that had been sweetened by storage at 1° C. the rate of respiration was determined by the activity of the complex of respiratory enzymes considered as a whole. Accordingly even prior to treatment with ethylene the rate of supply of sugar would have been sufficient for the cells to show their maximum respiratory activity at 15° C., *i.e.*, no stimulation was induced by treating such sweetened potatoes with ethylene.

It has long been known that the intrinsic respiratory activity of a given cell, tissue, or organ, may be altered by changes in external conditions; but it is not always easy to decide for certain whether such alterations are attributable to interference with respiratory enzymes or with organization resistance. Moreover, new concepts may have to be formulated to explain some of these effects. There is no doubt that the action of strong doses of poisons, such as hydrogen

cyanide, is to inhibit oxidation enzymes. The effects of weak doses may be more complex (see, *e.g.*, p. 284). The influence of narcotics may also be variable. Thus the rate of respiration of wheat seedlings is increased by weak doses of ether and decreased by stronger doses. Possibly the first effect is on organization resistance, and the second on the catalytic surface. Carbon dioxide narcotizes certain plant tissues and depresses respiratory activity. Kidd and West (*167*) have investigated the depressant effects of carbon dioxide on germinating seeds (*e.g.*, white mustard seeds, which they caused to enter into a state of secondary dormancy for a year after treating them with carbon dioxide) and stored fruits. A practical application of the knowledge gained has been the development in recent years of gas storage of fruits and vegetables. It appears that the storage lives of certain apples may be lengthened by keeping them in an atmosphere of ten per cent. carbon dioxide, ten per cent. oxygen and eighty per cent. nitrogen.

Injury to a given plant tissue often causes respiratory activity to increase. The phrase "wound stimulus to respiration" concisely describes but does not explain this interesting phenomenon. Moreover, mechanical shock without the infliction of injury may stimulate respiration. Audus (*8*) has reported that the rubbing and bending of leaves of certain xeromorphic and mesomorphic species of angiosperms (*e.g.*, cherry laurel, *Pelargonium sp.*) induced a sudden increase in the rate of CO_2 output. In all his experiments the increase was substantial; in most of them ranging from twenty to ninety-nine per cent., but in an experiment with Yucca a stimulation of 183 per cent. was observed. The stimulation gradually subsided during a period of two or three days. Plant material must necessarily be handled in setting up experiments for the measurement of respiration. Clearly it is imperative to allow time for the material to pass through the shock phase before measuring its normal respiration, and subsequently to avoid handling the material.

It has also been stated that roots reacting to the geotropic stimulus respire more actively than before stimulation, that the respiration of carpels is accelerated by pollination, and that CO_2 production and oxygen uptake of sea-urchin eggs increase rapidly immediately they are fertilized. Such increase possibly follows fertilization in all organisms.

Temperature. The experimental data that are graphically represented in fig. 37 show the typical effects of temperature on respiration. Samples from a population of four-day-old seedlings of the garden pea respiring at a constant rate of 25° C. were transferred to respiration chambers kept at different temperatures. At each temperature below 25° C. respiration gradually declined until it reached a more or less steady value. At each temperature between

25° C. and 35° C. respiration gradually rose to a steady value. It was calculated from the differences in these steady rates of respiration that, between the temperatures 0° C. and 35° C., Q_{10} (p. 234) for respiration lay between 2·0 and 2·5. Temperature coefficients of this order are what would be expected from van't Hoff's law on the effect of temperature on the rate of chemical processes.

Above 35° C. the curves were a resultant of two antagonistic processes. The stimulating effect of temperature on the chemical process of respiration continued. Thus we notice that there was a sharper initial rise of respiration at 45° C. than at 40° C. The other

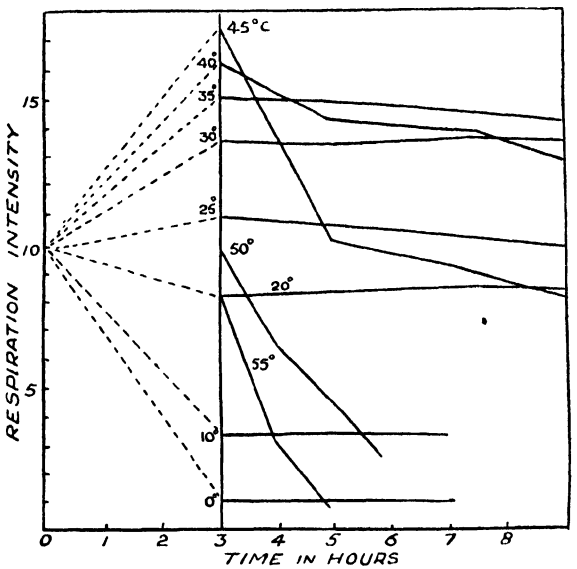


FIG. 37. The effects of temperature and the time factor on the rate of respiration of seedlings of the garden pea. (After Fernandez, see Stiles and Leach, *295*.)

effect was inhibitory owing to the fact that at temperatures above 35° C. respiratory enzymes are gradually inactivated. This general effect was responsible for the fall in respiration that occurred after prolonged exposure at all temperatures above 35° C. The higher the temperature the more rapidly did this inactivation progress: so rapidly, indeed, at temperatures greater than 50° C., that by the time the first measurements were made the respiration was less than it had been at the initial temperature of 25° C. Had it been possible to measure directly the rate of respiration immediately after the change from 25° C. to 50° C., it is probable that the rate at 50° C. would have been found to be more than double that at 25° C.

These results for the respiration of pea seedlings bring out clearly the relations between temperature and duration of exposure. Similar relations are also found in many other physiological processes. The idea of a time factor was suggested many years ago by F. F. Blackman. It corrected the long-held notion of a cardinal point called the optimum temperature for biochemical changes governed by thermolabile systems. In the last century it was believed that in addition to a minimum temperature below which and a maximum temperature above which a reaction would not occur, there existed for every reaction a definite optimum temperature at which the reaction proceeded most rapidly. But it will be seen for the respiration of pea seedlings, as Blackman earlier proved for the photosynthesis and respiration of green leaves, that the apparent optimum changes with the duration of the experiment. For the respiration of pea seedlings we note that after three hours the rate at 45° C. was greater than at 30° C., but after five hours the rate at 30° C. was greater than that at 45° C. In general it appears that the longer the period of exposure the lower was the apparent optimum. Similar results have been obtained for other physiological processes; hence the idea of a definite optimum for each process has been discarded. For processes governed by thermolabile systems we may state that the reactions they govern will be accelerated by increasing the temperature to the level at which inhibition sets in. Above this temperature a knowledge of the effects of duration of exposure, *i.e.*, of the time factor, must be gained before statements can be made.

Water content of cells. The results of experiments on seeds indicate that the water content of cells influences respiration. The rate diminishes during the drying-out of seeds to a low but measurable value for air-stored seeds containing about ten per cent. water, and increases when these seeds once more imbibe water. Water probably affects both the respiratory activity of protoplasm and the amount of soluble respiratory substrate.

Concentration of respirable substrate. There is direct and indirect evidence that at constant temperature the rate of respiration of a given tissue is governed by the concentration of the soluble respirable substrate. By feeding fungal hyphæ or etiolated leaves with various sugars, respiration has been enhanced. Further, it has been shown that green leaves respire more rapidly after a period of illumination, not because light has activated the respiratory mechanism, but because the concentration of carbohydrates is increased by photosynthesis.

The demonstration of the progressive decline in respiratory intensity shown by leaves starving in the dark (p. 271, *et seq.*), and of the relation between the rate of respiration and sugar concentration in the potato tuber (p. 275), has provided further evidence of the fact

that the concentration of respirable substrates may limit the rates of oxygen uptake and CO_2 production. Hanes and Barker (*116*) found that the sugar concentration rose in potato tubers when they were treated with weak doses of hydrogen cyanide, and attributed this rise to the activation of amylase by cyanide. The respiration of tubers sweetened in this way was more rapid than that of untreated tubers. It should be noted that higher concentrations of cyanide brought about a depression of respiration (see p. 280).

Oxygen concentration. In low concentrations of oxygen, carbon dioxide is produced both by aerobic and by anaerobic respiration. From his measurements of respiratory quotients, Stich concluded that anaerobic respiration is extinguished in most plant tissues when the oxygen concentration is raised to five per cent. Broadly, the effect of increasing the oxygen concentration from zero to the extinction point of anaerobic respiration depends upon (*a*) the ratio of respiration in the complete absence of oxygen to aerobic respiration (*i.e.*, I/N, p. 291), and (*b*) the rate at which anaerobic respiration is replaced by aerobic respiration. For apples, Parija found that anaerobic respiration is sometimes greater than aerobic CO_2 output, and gained evidence that the relatively high rate of CO_2 output shown by this fruit in pure nitrogen fell off rapidly on admitting oxygen until the extinction point of anaerobic respiration was reached at an oxygen concentration of about five per cent. Subsequently the CO_2 output of purely aerobic respiration rose and each additional increase in oxygen up to one hundred per cent. further stimulated respiration. Blackman attributed this stimulating effect of oxygen on aerobic respiration to its playing a part in the activation of hexoses prior to glycolysis (p. 307).

Knowledge of the way that oxygen concentration influences the rate of respiration will not be complete unless the rate of oxygen uptake is measured as well as that of CO_2 output. This is especially true when low concentrations of oxygen are used. In such experiments the rate of accumulation of ethyl alcohol should also be measured. A respiratory quotient greater than unity may make it probable that anaerobic respiration has set in ; the accumulation of ethyl alcohol renders this fact certain. We shall consider data obtained by experiments on the respiration of apple fruits, which appears to have been more intensively investigated than that of any other plant organ.

Since the value of the respiratory quotient of apples remains as about unity until anaerobic respiration sets in, the inference may be drawn that the rate of oxygen uptake in the experiments of Blackman and Parija fell gradually as the oxygen concentration was reduced to a value of about five per cent. Blackman stated that it had been proved in his laboratory that oxygen uptake is decreased by lowering

the oxygen concentration, and pointed out that unlike the CO_2 output this does not necessarily reach a minimum "in some one low concentration of oxygen." Thomas and Fidler (*310*) found the extinction point of anaerobic respiration for apples by determining the concentration of oxygen at which alcohol accumulation ceased. In their experiments anaerobic respiration in healthy full-grown apples was extinguished at all concentrations greater than three per cent. oxygen. When apples became senescent higher concentrations of oxygen were necessary. Fidler (*86*) showed that in old apples ethyl alcohol may accumulate even in air. The oxygen concentration in the intercellular spaces inside a bulky fleshy fruit is considerably lower than the external concentration (see p. 154). It must be admitted, therefore, that normal aerobic oxidative processes are maintained during most of the storage season by surprisingly low concentrations of oxygen.

Thomas and Fidler also measured the rate of total CO_2 output and their results supported the conclusion of Blackman and Parija that the minimum rate occurs at the extinction point of anaerobic respiration. At lower oxygen concentrations the total CO_2 output is compounded of anaerobic and aerobic CO_2 production. To simplify the discussion at this stage we shall assume that anaerobic respiration consists wholly in alcoholic fermentation (but see p. 288) and that ethyl alcohol and CO_2 are both end products. The anaerobic component may then be calculated from the rate of alcohol accumulation (see equation on p. 287). Clearly the residual carbon dioxide will represent aerobic respiration. The results of one experiment are given in table XIII.

TABLE XIII. *CO_2 output and alcohol accumulation in Bramley's Seedling apple in gas mixtures containing different percentages of oxygen. The results are expressed as mg. per* 100 *gm. fresh weight tissue per* 100 *hours.*

	Percentage Oxygen			
	21 per cent.	5·3 per cent.	2·0 per cent.	*Nil.*
Total CO_2 (exp.)	567	493	499	631
Alcohol / Total CO_2 (exp.)	*nil*	1 / 100	14 / 100	—
Anaerobic CO_2 (calc.)	*nil*	5	67	631
Aerobic CO_2 (calc.)	567	488	432	*nil*

In this experiment the extinction point of anaerobic respiration appears to have been rather higher than five per cent. oxygen. As

the concentration of oxygen was lowered the rate of anaerobic respiration increased to 631 and that of aerobic respiration decreased to zero.

Thomas and Foster (*unpublished work*) have found that the rate of oxygen uptake at low oxygen concentration, in which alcohol accumulation becomes a feature of carbohydrate catabolism, is much less than it is when apples are placed in air. It is highly probable that the rate of oxygen uptake (*i.e.*, the rate of aerobic respiration) continues to decline progressively as the oxygen concentration is gradually lowered to zero, and that the observed progressive increases in the rates of accumulation of ethyl alcohol and the liberation of carbon dioxide may be attributed to this decline. In terms of a theory to be discussed later (p. 300) we may explain these facts by stating that the production of ethyl alcohol and the liberation of carbon dioxide proceed at ever increasing rates when the oxidative activity of respiratory enzymes is depressed beyond the value associated, in healthy apples, with an external oxygen concentration of about three per cent. (the extinction point of anaerobic respiration). The maximum rates will be shown in the complete absence of oxygen.

It is probable that the respiration of many other fleshy fruits and of some other organs (*e.g.*, carrot roots, cotyledons of the vegetable pea) may respond similarly to that of the apple to changes in oxygen concentration. In 1940 Ranson (*unpublished work*) obtained evidence in support of this view, for rhododendron leaves and certain germinating seeds. It may well be that the rates of CO_2 output and of oxygen uptake by all tissues show a tendency to decline until, at some low oxygen concentration, anaerobic respiration sets in, and that the oxygen uptake of the residual aerobic respiration will continue to fall as the oxygen concentration is still further lowered. But there exists evidence, such as that obtained by Leach (*178*) in his experiments on certain seedlings, that the rate of CO_2 output by some organs placed under anaerobic conditions may be less than that shown by these organs when in air. This suggests that in these organs the rate of CO_2 output is progressively depressed as the oxygen concentration is gradually lowered to zero.

In recent years wider interest has been shown in the influence of oxygen concentration on respiration because of the discovery that respiratory intensity may affect the rates of certain vital processes, such as the absorption of mineral salts (p. 91). It appears that under natural conditions only exceptionally is the rate of supply of oxygen to respiring cells the factor that limits the rate of respiration, and, therefore, of dependent processes. We have no space here to discuss the exceptional instances, such as the respiration of swollen seeds with thick coats, of buds with thickly cutinized scales, of deep-

seated cells in the trunks of trees, or of the roots of plants growing in water-logged soils.

The depression of gaseous exchange in some of these organs may result in the accumulation of ethyl alcohol and low concentrations of acetaldehyde. Certain workers have considered the possible functional significance of such substances, but have arrived at conflicting conclusions. For example, Mazé has suggested that acetaldehyde may cause certain seeds to remain dormant, but Boresch thinks that the dormancy of the winter buds of some trees may be broken by the action of this aldehyde ; and Kakesita has asserted that acetaldehyde may promote the rooting of leaf-cuttings of Bryophyllum. It still remains possible, however, that dormancy and activity are in no way affected by such ethyl alcohol and acetaldehyde as may inevitably accumulate as a result of reduced oxidative activity.

F. Anaerobic Respiration

The fermentative component of anaerobic respiration. Experiments have shown that nearly all the tissues of higher plants produce carbon dioxide under anaerobic conditions, and that this anaerobic respiration usually consists in part at least of alcoholic fermentation.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 24\text{–}28 \text{ Cals.}$$

It should be noted that the energy liberated (24–28 Cals.) in the alcoholic fermentation of 1 gm.-molecule of hexose is much less than that (674 Cals.) set free by the complete oxidation of 1 gm. molecule into carbon dioxide and water. This will in part account for the superior functional value of aerobic respiration over anaerobic respiration. There may well be other and more important reasons for this superiority. Some of the energy liberated in alcoholic fermentation is converted into heat, but a fraction may be conserved by living cells in chemical bonds, *e.g.*, the active phosphate bonds in ATP (see p. 53).

We may attribute the fermentative component of anaerobic respiration to the action of zymase in living cells, seeing that this enzyme complex has been separated from a large number of different plant tissues. For example, a zymase preparation was obtained from beetroot, and the enzyme, under sterile conditions *in vitro*, acted on glucose giving alcohol and carbon dioxide in approximately the proportions required by the above equation. By weight the quotient (*rate of alcohol accumulation*)/(*rate of* CO_2 *output*), should work out at 1·04.

It has long been known that traces of other substances besides ethyl alcohol may accumulate when yeast is living anaerobically in solutions containing a fermentable sugar. Some of these substances (*e.g.*, glycerol, lactic acid, acetaldehyde) are produced by the zymase

cleavage of the sugar; others (*e.g.*, fusel oils) may result from the anaerobic nitrogen metabolism of the yeast cell. Moreover, it should be noted that the properties of zymase obtained from the higher plants are probably identical with those of the zymase of yeast. Accordingly it would not be surprising to find that in the tissues of the higher plants, placed under anaerobic conditions, other substances besides ethyl alcohol and carbon dioxide may accumulate. Actually it has been established (see p. 303) that in some tissues, but not in all, traces of acetaldehyde may accumulate, but no convincing evidence has yet been published of the accumulation, in the absence of fixatives or inhibitors, of any other intermediate products or by-products of zymase cleavage, or of products of the metabolism of cell components other than carbohydrates.

If anaerobic respiration consisted exclusively in alcoholic fermentation, the quotient *rate of alcohol accumulation/rate of* CO_2 *output* should work out at 1·04, as it does for zymase cleavage *in vitro*. Most of the published values for this quotient, examples of which are given in table XIV, are less than unity. It should be noted that these values are based on measurements of the total amounts of the carbon dioxide given off and of the ethyl alcohol that accumulates over an arbitrarily chosen period of time, which in some instances has been relatively short (*e.g.*, six hours) and in others relatively long (*e.g.*, six days). It remains to be proved that the rates of production of these substances march together during a whole period of anaerobiosis from the moment of transition from aerobic to anaerobic conditions.

TABLE XIV. *The quotient, alcohol accumulation/CO_2 output, in anaerobic respiration*

Cotyledons of edible pea	0·6–0·8
Carrot root	0·7–1
Apples	0·4–0·9
Orange	0·7
Grapes	0·7–0·9
Nasturtium leaves	0·2–0·4
Potato tubers	0–0·1

Certain general conclusions may be drawn from these results. Thus anaerobic respiration in the cotyledons of the edible pea, carrot roots, certain varieties of apple, and grapes, is predominantly alcoholic fermentation. In contrast, very little alcohol accumulates in the anaerobic respiration of potato tubers. In other varieties of apple and in nasturtium leaves fermentation accounts for less than half of the carbon dioxide liberated.

It appears that for a single tissue there is some variation in this quotient. Fidler (*87*) reported that for a given variety of apple in any one storage season the quotient might fluctuate widely about a mean value, but that this mean did not alter as the apples aged. The general drift of the charted values for the rate of accumulation of ethyl alcohol and that of CO_2 output ran parallel (fig. 38). The mean value might, however, vary from season to season. For example, the mean value for the Newton Wonder apple in 1930–31 was 0·9, and in 1932–33 it was 0·65. It should be noted that the rate of zymase cleavage may be limited by the concentration of sugar as well as by the activity of zymase. The effects of a shortage of sugar on the value of the quotient are better studied if leaves that have been starving in the dark are used as experimental material. Some evidence has been obtained at Newcastle upon Tyne that as starvation proceeds the quotient tends to decrease. In the light of the results of Yemm's experiments (p. 274) this would suggest that relatively high values of the quotient are to be related to the presence of relatively high concentrations of sugar, and that carbon dioxide may be produced as a result of anaerobic nitrogen

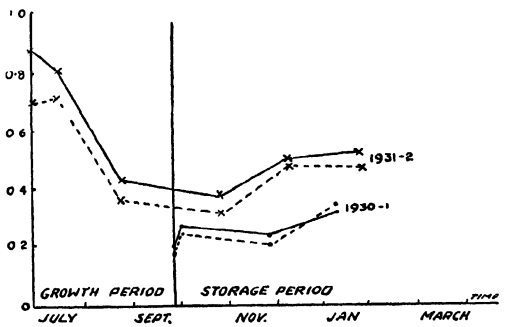


FIG. 38. Rates of CO_2 output (——), and of alcohol accumulation (- - - -) in Newton Wonder apples in nitrogen at 23° C., expressed as gm. substance per 100 gm. fresh weight during 100 hours (from Fidler, see text).

metabolism, when leaves suffering from a high degree of starvation are transferred from the dark in air to the dark in pure nitrogen. But clear evidence of decarboxylations resulting from anaerobic nitrogen metabolism has not yet been published.

Indeed we do not yet know of any other anaerobic process besides zymase cleavage in which carbon dioxide is produced. Substrates other than carbohydrates may be acted on; and carbohydrates may be cleaved by other enzymes than zymase. Accumulation of acetaldehyde, the only precursor of ethyl alcohol to be produced during decarboxylation, is never sufficient to account for low values of the quotient alcohol production/CO_2 production; but it should be noted that acetaldehyde and ethyl alcohol may possibly be further metabolized under anaerobic conditions giving rise to carbon dioxide or to organic products which have not yet been identified. Clearly, if this happens the rates of production of these substances would be greater than their rates of accumulation.

The progress and rate of the accumulation of ethyl alcohol and acetaldehyde. Thomas (*303*) appears to have been the first worker in this field to measure the progress of the accumulation of ethyl alcohol (*i.e.*, the progress of zymasis) in the tissues of the higher plants under anaerobic conditions.

His results for Newton Wonder apples respiring anaerobically at three different temperatures, are graphically represented in fig. 39. The main features to be noted here are (*a*) that until the apples were injured alcohol accumulated at the steady rates (expressed in mg. per hour per 100 gm. fresh weight tissue) of about 4·4 at 22° C., 1·8 at 15° C., and 0·6 at 1° C., and (*b*) that injury led to a slowing down, and death to the cessation of the accumulation of alcohol. It may be said that ethyl alcohol is produced because cells are living under anaerobic conditions, and is produced only as long as they remain alive. Zymasis is not a *post-mortem* phenomenon, neither is it necessarily a *pre-mortem* phenomenon; seedlings will grow,

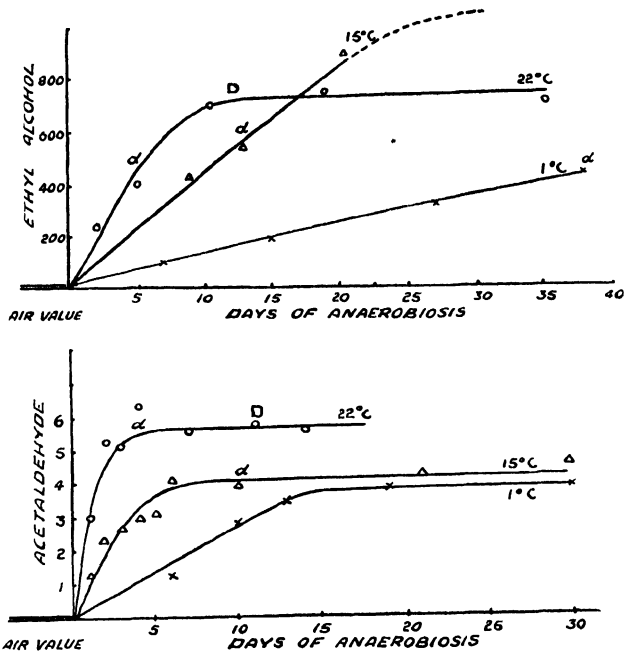


FIG. 39, (*a*) and (*b*). The accumulation of ethyl alcohol and acetaldehyde by Newton Wonder apples, expressed as mg. substance per 100 gm. fresh weight tissue (from Thomas, see text). Visible injury, *d.*; tissue dead, D.

and apples will remain healthy for many months when returned to air after short exposures to anaerobic conditions.

Other workers have calculated mean values for the rate of zymasis (which we shall represent by Z) from the mean rate of alcohol accumulation. For example, Boysen-Jensen, expressing rates as mg. alcohol accumulating per hour per 100 gm. fresh weight tissue, obtained a mean value of 8 for pea seedlings at temperatures of 18·9–20° C., of 6·3 for Tropœolum leaves in a four-hour experiment at 15° C., and from zero to 0·1 in a series of experiments of duration forty-seven to seventy-six hours at 22° C. on potato tubers.

Evidently little significance can be attached to such mean values when the rate of anaerobic respiration declines rapidly during the course of an

experiment. What we should then want to know would be the rates of alcohol accumulation at all times during anaerobiosis. It should be noted that rate curves can always be constructed from the data recorded in progress curves, and that by extrapolation the probable rate of alcohol accumulation at the moment of transition from anaerobic to aerobic conditions may be determined (*c.f.* Blackman's extrapolation described on p. 293).

Thomas (*loc. cit.*) also measured the accumulation of acetaldehyde in apples (fig. 39 (*b*)) during anaerobic zymasis. It will be observed that this substance ceased to accumulate before the rate of alcohol accumulation had been markedly retarded; and that the concentration of acetaldehyde present at any time was very small in comparison with that of ethyl alcohol. Similar results have since been obtained for other tissues which produce acetaldehyde anaerobically.

There is good evidence (see Kostytschew, *175*) that for a given organ of a selected species of plant the intensity of aerobic respiration and the rate of CO_2 output under anaerobic conditions march together. Fidler (*87*) made experiments during two seasons on the anaerobic respiration of Newton Wonder apples picked in a certain orchard in Kent, and his charted results in fig. 38 indicate that the rates of accumulation of ethyl alcohol and of anaerobic CO_2 output also march together. It will also be seen that the form of these charts is similar to the form of the curve in fig. 25, p. 210, showing the seasonal drift in the intensity of aerobic respiration in apples. When this intensity is relatively high, as it is in young growing apples, so also are the rates at which CO_2 is set free and ethyl alcohol accumulates under anaerobic conditions. In the early stages of storage all the rates are relatively low, but increase during the climacteric. A possible explanation of these relations is that the enzymic systems governing anaerobic respiration form part of the systems that control aerobic respiration. Alternatively, aerobic and anaerobic respiration may be independently controlled, but the rates of both processes may be governed by the general metabolic vigour of the cells. Kidd and West (*168*) have shown by their experiments on aerobic respiration that this vigour may vary from year to year, as well as during the development and storage of apples. This fact is also illustrated by the results of Fidler's experiments, since he found that the intensity of anaerobic respiration was less in the season 1930–31 than in the season 1931–32.

I/N quotients. For over fifty years attempts have been made to determine the quotient obtained when the rate of anaerobic respiration (often denoted by the letter I, which stands for intramolecular respiration [1]) is divided by the rate of aerobic CO_2 output (often denoted by the letter N, which stands for normal respiration); but, for reasons that will be given below, the results of earlier researches are of doubtful value. We mention, however, that this quotient was obtained by dividing a number representing the mean rate of CO_2 output by a given tissue in nitrogen or hydrogen, *i.e.*, in the absence of oxygen, by a number representing the mean rate for the same tissue placed in air. Fluctuations in rate during the course of

[1] This term was in general use some years ago for respiration in the absence of oxygen, but became obsolete when it was realized that aerobic respiration also results from intramolecular change The old symbols are, however, retained for the convenience of the reader when he refers to monographs on respiration.

the experiments were not taken into consideration. Now, respiration usually remains fairly constant during short periods of a few hours in air at constant temperature, so a significant value for N can be obtained by dividing the total amount of carbon dioxide liberated during a period by the number of hours. But, under anaerobic conditions, the rate of CO_2 output often alters rapidly. Thus it was found that the average hourly rate for white mustard seedlings was, during the fourth hour of anaerobiosis, only one-quarter of the rate during the first hour, and consequently the I/N quotient fell as the experiment proceeded. What is usually wanted in order to express this quotient is a value representing I before any change in anaerobic respiration has occurred. This means that a number must as a rule be obtained which represents I immediately after anaerobic conditions have been imposed on the plant tissue under experiment. In recent years F. F. Blackman and Parija have pointed a way to determining such a number. These workers asserted that for Bramley's Seedling apple they have actually succeeded in measuring I (they call this nitrogen respiration or N.R.) and N (they call this oxygen respiration, or O.R.), as if the given apple were respiring simultaneously in nitrogen and in air. Having overcome the difficulty of the fluctuation of I with the duration of anaerobiosis they maintained that from the quotient I/N (or N.R./O.R.) so obtained inferences may be made about the effect of oxygen on the rate of CO_2 output.

A Bramley's Seedling apple in which the rate of aerobic respiration was slowly but steadily decreasing, was transferred to nitrogen at a time R_1, when its aerobic respiration was equal to O_1R_1 (see fig. 40). Further measurements of CO_2 output were made at three-hour intervals, and it was found that the rate at which carbon dioxide left this bulky fruit was greater after three hours' anaerobiosis than before the apple was placed in nitrogen. This rate increased for a further six hours, reached a value m_1, and then decreased at an apparently steady rate along m_1N_2. After forty-eight hours in nitrogen, when the anaerobic respiration was equal to N_2R_2, the apple was again exposed to air, and the first measurement showed that the rate of liberation of carbon dioxide had decreased. Subsequently respiration fell to a minimum value m_2, which was lower than the value that would probably have been obtained at the time of measurement had the apple never been through a nitrogen experience. The rate of this aerobic respiration then increased until it once more reached the air line at *l*. Apart from minor fluctuations, aerobic respiration then followed the air line, *i.e.*, it decreased at approximately the same rate as before the nitrogen experience, and Blackman concluded that " the nitrogen experience is at last, after a couple of days, a thing of the past which has left no permanent effect."

The results, which are graphically represented in fig. 40, show that by using the method of the older workers to evaluate I/N we should obtain different numbers were we to make comparisons of I and N first over short and then over long periods. Moreover, the I/N quotient found after transferring from air to nitrogen would be different from that found after re-transferring from nitrogen to air. Blackman's method, however, permits comparisons of I and N to be made at one and the same time R_1 and at one and the same time R_2, although, actually, only N at R_1 and I at R_2 are determined experimentally. I at R_1 and N at R_2 were found by extrapolation.

Blackman postulated that the rate of CO_2 output by the flesh tissue at once increased when the apple was transferred from air to

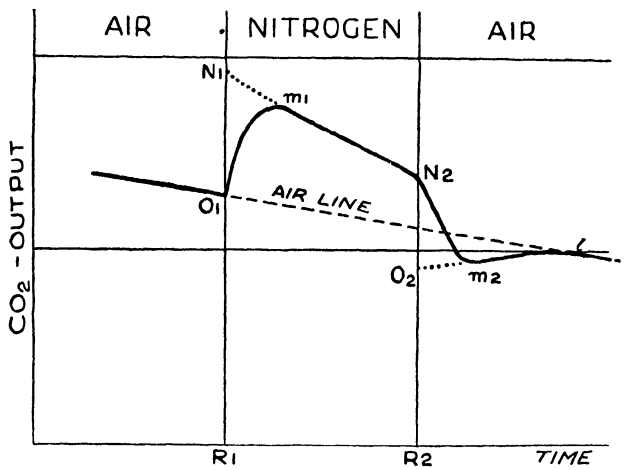


FIG. 40. Graphical representation of the aerobic and the anaerobic respiration of a Bramley's Seedling apple. The drawing is based on one of the figures given by Blackman (*33*).

nitrogen, and that afterwards the rate of anaerobic respiration steadily decreased. It will, however, be observed from O_1m_1 in fig. 40 that what was actually first found by measurement was a gradually rising rate of liberation of carbon dioxide through the skin of the apple. Blackman pointed out that the skin, which is covered by a thick cuticle pierced but sparsely by lenticels, would offer a considerable resistance to the diffusion of gas from the intercellular spaces within. Hence he concluded that for purely physical reasons it takes several hours for the full effect of changes in tissue respiration, whether anaerobic or aerobic, on the CO_2 content of an apple to become equilibrated with the rate of diffusion of carbon dioxide through the lenticels. The curve between the points O_1 and m_1 in fig. 40 represents the fact that respiration had increased on transferring from air to nitrogen. But we can deduce nothing else, as

equilibrium between CO_2 production and CO_2 escape was not reached until the rate of escape had risen to m_1. Subsequently along m_1N_2 there was a steadily falling rate of escape,[1] which was a true expression of the falling rate of anaerobic respiration of the flesh tissue. Blackman inferred, and this inference is the crucial point in his analysis, that anaerobic respiration had, since its inception, been decreasing at this same steady rate. Hence he concluded that the initial value of anaerobic respiration could be obtained by continuing the curve N_2m_1 back to N_1, *i.e.*, whereas the curve O_1m_1 represents CO_2 escape as measured by experiment, the dotted curve N_1m_1 represents the actual CO_2 given off by the respiring cells. Plainly he had achieved his object of getting numbers for the aerobic and anaerobic respiration of a single apple at one and the same time; for at the time R_1 aerobic respiration was by experiment found to be O_1R_1, and anaerobic respiration was evaluated as N_1R_1 by extrapolation from the curve obtained by experiment for subsequent anaerobic respiration.

The events that occurred on returning the apple to air from nitrogen were subjected to a similar form of analysis. At the time R_2 it was supposed that when aerobic respiration replaced anaerobic respiration the rate of CO_2 output by the flesh tissue immediately decreased. But owing to the barrier to diffusion imposed by the skin, some of the residual carbon dioxide produced during the last hours of anaerobiosis would still be present in the intercellular spaces. Hence, immediately after returning the apple to air, the CO_2 escape was higher than the actual CO_2 production by aerobic respiration, and N_2m_2 virtually represents the escape of this residual carbon dioxide. Equilibration between CO_2 production by aerobic respiration and CO_2 escape began at m_2, and m_2l represents the trend of the gradual increase in aerobic respiration that occurred from its initial low value at the time R_2. A number (O_2R_2) for the actual aerobic respiration of the flesh tissue at the time R_2 was obtained by continuing the curve lm_2 back to O_2. Thus at the time R_2, Blackman found by experiment the number N_2R_2 for anaerobic respiration, and by extrapolation from a curve obtained by experiment the number O_2R_2 for aerobic respiration. He therefore succeeded in evaluating the I/N quotient for a single apple at two distinct times, and found that this quotient was the same whether it was calculated for the time R_1 or the time R_2, *i.e.*, $N_1R_1/O_1R_1 = N_2R_2/O_2R_2$. For the season in which Blackman's experiments were carried out the I/N quotient for Bramley's Seedlings of a quick-ripening class worked out at 1·8, and for a slow-ripening class at 1·5.

[1] We see that, during this period, anaerobic respiration decreased more rapidly than aerobic respiration, for m_1N_2 declines more steeply than does the airline (*cf.* footnote, p. 307),

The conservation of carbon. (i.) ***I/N quotients greater than unity.*** If the rate of CO_2 output is a measure of respiratory catabolism, it may be inferred, without further consideration of metabolic events, that in those tissues which give I/N quotients greater than unity *the presence of oxygen tends to conserve respirable substrates, i.e.*, to conserve carbon combined in oxidizable organic compounds. Such conservation is clearly illustrated by Blackman's results for Bramley's Seedling apple. Using Blackman's method other workers have obtained quotients greater than unity for tomatoes (Gustafson, *105*), cherry laurel leaves and carrot roots (see Turner, *315*), and for rhododendron leaves (Ranson, unpublished work). Although doubt still exists concerning the significance of I/N quotients recorded by earlier workers (see p. 292) quotients greater than unity found for certain tissues (*e.g.*, germinating peas and beans, green grapes) provide further evidence of the conservation of carbon in the above described sense. Had Blackman's method been used even higher quotients might have been obtained for such tissues. Boysen-Jensen (*46*) implied that substrate is conserved as a result of the absorption of oxygen when he pointed out that in these instances the rate of carbohydrate destruction must have been greater under anaerobic conditions than it was in air.

Meyerhof has, as a result of his work on respiration and glycolysis in yeast and in muscle tissue, strongly asserted that aerobic respiration should be regarded as a process that conserves the plastic substances in cells. It is true that organic metabolites are lost during aerobic respiration, but Meyerhof maintained that the loss is less than when aerobic respiration is stopped by cutting off the oxygen supply.

In the apple and in other plant tissues in which ethyl alcohol accumulates during anaerobiosis, the amount of respirable substrate that disappears is considerably greater than is represented by the results of measurements of CO_2 output. In order to evaluate the effect of oxygen in conserving carbohydrates in apple cells Blackman expressed sugar loss in the presence and absence of oxygen in terms of carbon units, taking the rate of carbon loss by aerobic respiration as unity. He assumed that anaerobic respiration consisted wholly in alcoholic fermentation as represented by the equation on p. 287; and, accordingly, he evaluated the rate of carbon loss by alcohol production as double that by N.R., which he determined experimentally.

Rate of carbon loss by O.R. in air = 1. . . . . (1)
Rate of carbon loss by N.R. in nitrogen = 1·3
Rate of carbon loss by alcohol production in nitrogen = 2·6.
Rate of total carbon loss in nitrogen = 3·9 . . . . (2)

From (1) and (2) it follows that, on the average, for every carbon unit lost by aerobic respiration, 2·9 carbon units, which would in the absence of oxygen be changed to alcohol or carbon dioxide, are conserved as respirable material. This is rather less than the conserving effect of oxygen found by Meyerhof for yeast and muscle tissue.

According to Fidler (*87*) only eighty per cent. (approximately) of the nitrogen respiration (*i.e.*, of the I) of the Bramley's Seedling apple results from alcoholic fermentation. It is possible, therefore, that Blackman slightly overestimated carbon loss attributable to the formation of ethyl alcohol; but even if he had greatly overestimated this loss his general conclusions would not have been effected, since I was so much greater than N in the apples he used. The desirability of determining experimentally the rate of alcohol accumulation as well as that of anaerobic CO_2 output,

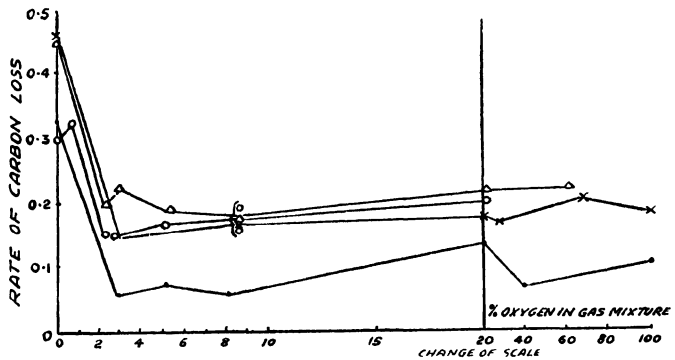


FIG. 41. Records of four similar experiments, showing the influence of oxygen concentration on the rate of carbon loss by Bramley's Seedling apple, at 23° C. Results expressed in gm. carbon per 100 gm. fresh weight tissue per 100 hours. (From Thomas and Fidler, see text.)

gave a clear lead to further work. On the basis of their measurements of these two rates Thomas and Fidler (*310*) constructed charts showing the influence of varying oxygen concentrations on the rate of carbon loss (fig. 41). Their results confirmed Blackman's conclusion that the minimum rate of carbon loss occurs at the extinction point of anaerobic respiration, *i.e.*, at a concentration of about 3 per cent. oxygen. These charts provide clear evidence that respirable substrates in apples are conserved by increasing the concentration of oxygen from zero to 3 per cent. With further increase the rate of aerobic CO_2 output continues to rise (p. 285), and consequently, the rate of carbon loss is progressively augmented. For the four experiments the approximate average rate of carbon loss under anaerobic conditions was 0·38; at the extinction point it was 0·14; and in air it was 0·18. Evidently, at the extinction point, for every unit of carbon produced in the carbon dioxide given off in aerobic respiration 1·7 units were conserved. In air the conserving effect of aerobic respiration was relatively less; only 1·1 units were conserved for every unit lost in the CO_2 liberated by the fruit.

(ii.) *I/N quotients of unity or less.* Although suggestive hypotheses consonant with experimentally determined values of CO_2 output may be put forward when I/N quotients are found to be unity or less than unity, the present writer maintains that no conclusions about the possible conserving effect of oxygen can be legitimately drawn unless simultaneous measurements are made of the production of substances other than carbon dioxide or of the consumption of respirable substrates.[1]

It was pointed out by Pfeffer that if aerobic respiration were wholly replaced by alcoholic fermentation on transferring plant tissue from air to anaerobic conditions, and if the rate of sugar destruction were not altered, the I/N quotient should work out at 0·33. This is because only one-third of the carbon lost in alcoholic fermentation occurs in the carbon dioxide set free, the remaining two-thirds are found in the ethyl alcohol that accumulates in the tissue. Some of the quotients obtained by earlier workers were less than 0·33, but doubt exists concerning their significance (p. 295). We wait therefore for confirmation of these low quotients by workers who use Blackman's method of extrapolation or some other method by which I may be evaluated immediately anaerobic respiration begins.

Using modern methods, Leach (*178*) determined I/N quotients for certain germinating seeds at the moment of transition from anaerobic to aerobic conditions. The values he obtained for buckwheat were 0·36, 0·31, 0 36, and 0·37. Since the average value was only slightly greater than 0·33 he inferred that at the moment of transition the complete oxidation of hexose to carbon dioxide and water was replaced by its anaerobic breakdown to carbon dioxide and alcohol, without alteration in the rate of sugar destruction, thus implying that oxygen exerted no conserving effect on respirable substrate in the buckwheat seeds that he used. For Zea Mays and the sweet pea the quotients found were less than 0·33. Even if in these plants also aerobic respiration were wholly replaced by alcoholic fermentation, the inference to be drawn would be that the rate of substrate loss in air was greater than that under anaerobic conditions, *i.e.*, *under the conditions of these experiments the presence of oxygen did not retard but accelerated the rate of carbon loss.* The quotients obtained for sunflower varied from 0·42 to 0·58, for *Cucurbita pepo* from 0·37 to 0·43, and for castor oil from 0·40 to 0·72. It is clearly possible that oxygen uptake may lead to retardation in the rate of carbon loss by these plants. But no definite conclusions can be legitimately drawn until further quantitative information has been obtained about other metabolic events occurring under anaerobic conditions.

[1] The possible aerobic consumption of CO_2 as a metabolite (see p. 329) will have to be taken into account for tissues which can bring about such changes as those represented in fig. 1.

A step in the right direction is to take into account the rate of alcohol accumulation as well as of anaerobic CO_2 output. Ideally the rate of carbon loss by aerobic CO_2 output should be compared with the rate of total carbon loss by the anaerobic production of ethyl alcohol as well as of carbon dioxide at the moment of transition from aerobic to anaerobic conditions (see pp. 290 and 292). Ranson made, some years ago at Newcastle, such measurements and calculations, and compared carbon loss under anaerobic conditions with that at the extinction point of aerobic respiration as well as with that in air. It is hoped that his results will be published soon. At present, since data for rates of alcohol accumulation (Z, see p. 290) are still scanty, consideration will be given to data for values of I/N and Z/I obtained simultaneously in experiments by Boysen-Jensen (*46*) on various tissues.

If the rate of aerobic CO_2 output is N, that of carbon loss in air will be 12N/44. Similarly a rate of anaerobic CO_2 output of I implies a rate of carbon loss of 12I/44; and an average rate of accumulation of ethyl alcohol (C_2H_5OH) of Z means a rate of carbon loss of 24Z/46. Evidently, if there are no other products of aerobic and anaerobic respiration besides carbon dioxide and ethyl alcohol, or traces of substances (*e.g.*, acetaldehyde) which may be included in an "alcohol number," a necessary condition for demonstrating that oxygen conserves respirable substrate is that

$$[(12I/44 + 24Z/46) - 12N/44] \text{ should be positive,}$$

$$\textit{i.e.,} \left[(1 + 1{\cdot}91Z/I) - \frac{N}{I}\right] \text{should be positive.}$$

It should be noted that in this argument no assumption is made about the chemical nature of the respiratory substrate that gives rise to aerobic and anaerobic carbon dioxide.

Inspection of column 5 in table XV shows that the differences of the values in columns 2 and 4 provide clear evidence of conservation for the succulent fruits blue and green grapes (*cf.* evidence for apple and tomato, p. 295); and also for carrot roots and pea cotyledons. In potatoes in which no alcohol accumulated the rate of carbon loss appears to have been the same under aerobic and anaerobic conditions. It is probable that the methods of measuring ethyl alcohol were not sufficiently accurate for much reliance to be placed on the small positive differences recorded in column 5 for the tubers that produced alcohol. We note the possibility, however, that substrate may be conserved to a slight extent by the influence of oxygen on the carbohydrate catabolism of such tubers. In the seedlings of white mustard, and, possibly, in nasturtium leaves, substrate loss was greater in air than under anaerobic conditions. We again point out, however, that we have no published data for a comparison between

TABLE XV. *Analysis of data obtained by Boysen-Jensen to illustrate a method of testing whether oxygen conserves respirable substrate (see text)*

	1 $\frac{I}{N}$ Experimental	2 $\frac{N}{I}$ Calculated	3 $\frac{Z}{I}$ Experimental	4 $\left(1+\frac{1{\cdot}91Z}{I}\right)$ Calculated	5 $\left[\left(1+\frac{1{\cdot}91Z}{I}\right)-\frac{N}{I}\right]$
Green grapes	1·2	0·84	0·81	2·55	+ 1·71
Blue grapes	0·74	1·35	0·88	2·68	+ 1·33
Carrot root	1·1	0·91	0·91	2·74	+ 1·83
,, ,,	0·58	1·72	0·69	2·32	+ 0 60
Pea cotyledons	0 83	1·20	0·65	2·24	+ 1·04
Nasturtium leaves	0·55	1·82	0·24	1 46	− 0·36
Potato tubers	1 0	1·0	zero	1	zero
,, ,,	1 1	0·91	0·2	1·38	+ 0·47
,, ,,	0 73	1·37	0·33	1 63	+ 0 26
White mustard seedlings	0·18	5·56	0·5	1·96	− 3 60

the rates of loss under anaerobic conditions and at the extinction point of anaerobic respiration.

It is emphasized that the burden of this section has been to assess such evidence as exists of the conserving influence of oxygen, and that no regard has been paid to possible ways in which this influence may operate. The consideration of these ways will form the subject-matter of a later section (p. 307).

G. The Development of Recent Views concerning the Connection between Aerobic and Anaerobic Respiration

The hypothesis that labile products of zymase cleavage may be oxidatively consumed. (i.) *Historical.* The idea put forward by Pflüger that anaerobic cleavage is the first step in the aerobic respiration of animal tissues was developed for plant respiration by Pfeffer late in the last century. Pasteur and his pupils had earlier established that many plants when deprived of oxygen produce ethyl alcohol as well as carbon dioxide, and Pfeffer suggested that the aerobic respiration of such plants might proceed in two stages, viz., (1) anaerobic cleavage with the formation of ethyl alcohol and carbon dioxide, and (2) oxidation of ethyl alcohol with the formation of further carbon dioxide and of water. This simple hypothesis was relinquished for several reasons, of which the most potent was and still is the fact that such experiments as have been performed indicate that plant cells either cannot oxidize ethyl alcohol, or, if they can, do so far less readily than they oxidize the carbohydrate substrate from which the alcohol is derived. It would take us too far were we to attempt to follow the vicissitudes of Pfeffer's hypothesis during the closing years

of the nineteenth century. Pfeffer himself abandoned his hypothesis for reasons which he doubtless would not later have considered valid. Those physiologists who decided that aerobic and anaerobic respiration were in no way connected, considered the latter either as a pathological phenomenon with no biological meaning or as a biological adaptation when it took place under those rare natural conditions in which there is a shortage of oxygen (see Kostytschew, *175*).

Many plant physiologists, however, continued to maintain that Pflüger's idea provided the key to the elucidation of the chemistry of aerobic respiration. This view was strengthened by experiments which appeared to show that aerobic respiration could sometimes be stimulated by subjecting certain plant tissues to anaerobic conditions

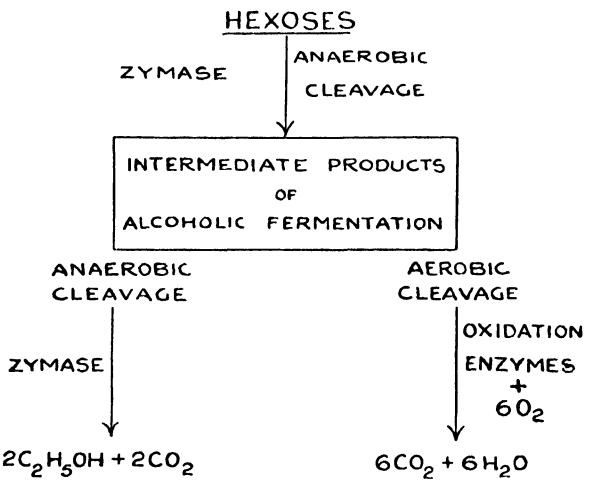


FIG. 42. Schematic representation of Kostytschew's views concerning the chemistry of respiration (see text).

for short periods. On returning the tissues under experiment to air, aerobic respiration began again at a higher rate than before the period of anaerobiosis. The increase in respiration was attributed to the accumulation during anaerobiosis of cleavage products that were more readily oxidizable than the usual respiratory substrates. There are other possible explanations (*e.g.*, the stimulation of respiration by the cleavage products, without their being oxidized), and there is much scope for further experiment.

The separation of zymase first from yeast and later from the cells of higher plants, and the study of the properties of this enzyme *in vitro*, paved the way for the modifications made in Pfeffer's hypothesis by the Russian physiologists, Palladin and Kostytschew, in order to meet the difficulties that had led to its being set aside by

so many physiologists. They came to the conclusion that alcoholic fermentation proceeds in stages, and that it is not ethyl alcohol that is produced and oxidized in aerobic respiration, but some labile intermediate product of zymase cleavage. This modified hypothesis is schematically represented in fig. 42.

It will be observed that this hypothesis only takes into account the fermentative component of anaerobic respiration. Even at the present day there is insufficient experimental data to warrant the extension of the hypothesis so as to include anaerobic cleavage processes other than those governed by zymase.

The evidence that was gathered and the inferences that were made before this hypothesis was formulated may be summarized thus :

(1) Zymase is widely distributed in the cells of higher plants. Hence the fermentative component of anaerobic respiration may be attributed to the activity of this enzyme.

(2) Studies on zymase *in vitro* had shown that the activity of this enzyme was not reduced in the presence of molecular oxygen. Hence it was reasonable to suppose that in living cells undergoing aerobic metabolism, carbohydrates would be acted on by zymase.

(3) Ethyl alcohol, however, is never present in significant amounts in the cells of higher plants living under aerobic conditions. Hence it follows that if zymase cleavage occurs under these conditions either ethyl alcohol or one of its precursors must be consumed as soon as it is produced. Clearly this must be an oxidative consumption, seeing that ethyl alcohol accumulates when the oxygen supply is cut off. Both Palladin and Kostytschew attributed this consumption entirely to the oxidation of this intermediate compound to give carbon dioxide and water.

(4) The Russian physiologists agreed that such evidence as was available from feeding experiments (p. 216) indicated that ethyl alcohol is either not consumed in air by the cells of higher plants, or, if it is consumed, not at a sufficient rate to warrant its being regarded as an intermediate product of aerobic respiration. Hence they concluded that oxidation is incident upon one or more of the precursors of ethyl alcohol in the chain of intermediate products formed in zymase cleavage.

In support of his hypothesis Kostytschew professed to have demonstrated that a mixture of products formed by the fermentation of sugar by yeast stimulated the aerobic respiration of wheat seedlings. He realized, however, that further consideration of the hypothesis would have to be deferred until more was known about the chemistry of zymase cleavage in yeast and the higher plants.

(ii.) *Intermediate metabolism in anaerobic respiration.* We have already pointed out (p. 49) that for the zymase of yeast this knowledge is now very considerable. Moreover, there exists evidence that zymase derived from the higher plants possesses properties similar to those of yeast zymase. It is probable therefore that when higher plant zymase is acting on hexoses *in vitro*, or when anaerobic zymasis is taking place in the higher plants, the biochemical changes that lead to the production of ethyl alcohol are similar to those which

occur when hexoses are fermented by yeast zymase, *i.e.*, the sequence is hexose, hexosephosphates, triosephosphates, phosphoglyceric acid, phosphopyruvic acid, pyruvic acid, acetaldehyde, ethyl alcohol. Substances such as glycerol, methyl glyoxal, lactic acid, and acetic acid, may be produced as by-products.

The older evidence concerning phosphorylation in the higher plants has been discussed by Onslow (*214*). Our knowledge of it is growing but is still incomplete. It is reasonably certain that fructofuranose diphosphate (see p. 276) is the substance that undergoes glycolysis, but it has not yet been proved that glucose-6-phosphate and fructose-6-phosphate are intermediate products in the conversion of glucose into the diphosphate (*cf.* glycolysis in yeast, fig. 2). Inasmuch as Hanes has found phosphoglucomutase and phosphorylase in association in peas and potatoes we may infer that glucose-6-phosphate can be produced from starch in the higher plants.

Turning to triosis and reactions subsequent to it, we recall that Kobel and Neuberg (*171*) reported that enzyme preparations from germinating soya beans can convert hexose diphosphate into triosephosphates, but shall confine our attention here to the results of investigations carried out by several investigators at Oxford on the mechanism of glycolysis by barley saps. These showed a negligible oxygen uptake; accordingly the results throw light on stages in anaerobic glycolysis. The knowledge gained by 1941 was summarized by James and Bunting (*153*), and further evidence has been reported by James, Heard, and James (*155*).

It has been established that organic phosphates are present in barley seedlings, and that barley can promote some of the reactions depicted in fig. 2. In a number of the experiments the accumulation of pyruvic acid in sufficient amounts for detection as the 2 : 4 dinitrophenylhydrazone was used as an index of the occurrence of glycolysis similar in kind to that taking place in alcoholic fermentation. To retard the decarboxylation of pyruvic acid, the enzyme carboxylase was poisoned by 1-naphthol-2-sulphonic acid. Little or no pyruvic acid was found after incubating for forty-eight hours at 30° C. barley saps which contained hexoses in low concentrations and lacked adenylic acid, but "readily identifiable quantities" were produced when sucrose or glucose and adenylic acid were added together. Traces were detected when adenylic acid was added alone, coming, it was inferred, from the hexoses in the sap. The general conclusion drawn was that for pyruvic acid to be produced in any experiment, conditions must be favourable for the phosphorylation of hexose in the sap or of such sugars as might be added. It was confirmed that the pyruvic acid was derived from phosphorylated sugar and its products when, even in the absence of adenylic acid, it accumulated in reactions occurring after hexosediphosphate or phosphoglycerate had been added to barley sap. Neither of these reactions took place in the presence of sodium fluoride, which is known to block the conversion by yeast of phosphoglycerate to pyruvic acid (p. 58), but when hexosediphosphate was the substrate added, a substance having the properties of phosphoglycerate accumulated. In a further application of the method

of the differential inhibition of enzymes (p. 215), a substance having the properties of triosephosphate was detected after barley sap had been incubated at 30° C. for twenty-four hours with hexosediphosphate in the presence of iodoacetate, which inhibits the oxidation by yeast of triosephosphate to phosphoglycerate (p. 57). A fixation method (p. 214) also provided evidence that triosephosphate is produced during glycolysis by barley sap. In the presence either of hydrogen cyanide or of sodium bisulphite, both of which can combine with aldehydes, the yield of triosephosphate was much greater than in the experiment with iodoacetate. Clearly, the cumulative evidence justified the conclusion that phosphorylative glycolysis in barley appears to be similar to that in yeast (fig. 2).

The application of methods described on pp. 213–19 has provided substantial evidence in favour of the view that acetaldehyde is the immediate precursor of ethyl alcohol, and that it is produced by the decarboxylation of pyruvic acid :—

(1) Under anaerobic conditions traces of acetaldehyde may accumulate with ethyl alcohol. This fact was discovered by Kostytschew in his experiments on the anaerobic respiration of poplar inflorescences. Similar reports have since been made by Thomas for apples, several other fleshy fruits, and other organs (*e.g.*, carrot root), Neuberg and Gottschalk for pea meal and bean meal, and by Gustafson for shoots of *Opuntia spp.*

(2) Thomas has discovered that zymasis in the presence of oxygen may be induced in such organs of the higher plants as produce ethyl alcohol anaerobically, by inhibiting oxidation enzymes with low concentrations of hydrogen cyanide or hydrogen sulphide, or by subjecting the organs to the action of high concentrations of carbon dioxide. These forms of zymase cleavage have been described as HCN-, H_2S-, and CO_2-zymasis. Experiments have been performed on fleshy fruits, carrot roots and other underground storage organs, germinating peas and barley, seeds and pods of peas and beans, and leafy twigs and inflorescences of a number of plants. In general, higher concentrations of acetaldehyde and lower concentrations of ethyl alcohol are developed during these forms of zymasis than during anaerobic zymasis. A possible explanation is that oxygen, when present, competes with acetaldehyde as a hydrogen acceptor in systems involving alcohol dehydrogenase.

(3) In parallel experiments on pea meal, acting anaerobically on a dilute solution of glucose, Neuberg and Gottschalk found that 164 mg. of acetaldehyde were fixed (p. 215) when sodium sulphite was added to the fermenting mixture, while in the absence of sulphite only 5 mg. accumulated. The amount of alcohol formed was reduced from 3,000 mg. to 300 mg., partly owing to the fixation of acetaldehyde and partly to the depression of zymase activity by the sulphite. This experiment provided strong presumptive evidence that acetaldehyde is the immediate precursor of ethyl alcohol in the anaerobic respiration of the higher plants.

(4) It appears now to be firmly established that carboxylase exists in the tissues of the higher plants (see James and Norval, *156*) ; and evidence has been obtained by Bunting and James that this enzyme, like its counterpart in yeast, may be resolved into an insoluble apoenzyme and a soluble coenzyme, which is a diphosphate of vitamin B_1 (p. 445). If the enzyme forms part of the zymase complex, we may infer that acetaldehyde and carbon dioxide are produced anaerobically by the decarboxylation of pyruvic acid.

(5) If the holocarboxylase is present, any substance giving rise to pyruvic acid might be a source of acetaldehyde and carbon dioxide. James and Bunting (*153*) reported that, in the absence of a carboxylase inhibitor, the CO_2 output of barley sap may be augmented by the addition of pyruvic acid, and to a less extent by that of hexosephosphate or phosphoglycerate. Since barley sap can convert these phosphate esters into pyruvic acid, it was concluded that this acid was formed as an intermediate product, and that, by being decarboxylated, it gave rise to the increased CO_2 output. This conclusion was supported when it was shown that in the presence of sodium fluoride, which blocks the conversion of phosphoglycerate into pyruvic acid, no extra CO_2 was produced when hexosediphosphate was added to barley juice. Inasmuch as it was not reported by James and Bunting whether acetaldehyde or ethyl alcohol accumulated in their experiments, it is not yet clear to what extent glycolysis effected by the enzymes in barley juice differs in its later stages from the zymasis occurring in barley cells living under anaerobic conditions.

(6) Kidd and Trout have reported that when acetaldehyde in relatively high concentration is fed to the orange or the apple, ethyl alcohol is produced. If this alcohol production results from the reduction by alcohol dehydrogenase of the added aldehyde, and if the cells of higher plants behave like those of yeast (fig. 2), cozymase would be involved, and the reduction would be coupled with the oxidation of triosephosphate by its specific dehydrogenase.

(iii.) *Intermediate metabolism in aerobic respiration.* Returning to the consideration of Kostytschew's schema for aerobic respiration, we must next record such evidence as now exists of the production by higher plants respiring in air of ethyl alcohol and of intermediate metabolites of zymase cleavage, and of the possible aerobic oxidation of some of these substances. Actually very little consideration has been given to the aerobic production and consumption of substances other than ethyl alcohol, acetaldehyde, and pyruvic acid ; and even for these substances no conclusive evidence has been obtained. A few statements may, however, be made.

(1) It has been reported that ethyl alcohol and acetaldehyde may accumulate aerobically in low concentrations in certain plant organs (*e.g.*, many ripening fruits, carrot roots, leaves of succulent plants, catkins of hazel and poplar) in which there is no reason to suppose that there is a shortage of oxygen in the vicinity of respiring cells. As far as is known acetaldehyde has never been found without ethyl alcohol. This evidence, which for some of the organs requires substantiation, would be consonant with the view that these substances are intermediate products of aerobic respiration. Alternatively the facts could be explained by supposing that a precursor of acetaldehyde undergoes aerobic respiration, but that the mechanism is not perfect and a fraction escapes oxidation and is further changed by the zymase complex, which retains its full activity in spite of the presence of oxygen. It should be noted as a further possibility that acetaldehyde, and consequently ethyl alcohol, may arise from vegetable acids and not from sugars in fleshy fruits and succulent leaves.

(2) Clearly the fact that both acetaldehyde and ethyl alcohol may accumulate when oxidation enzymes are inhibited either by depriving a

tissue of oxygen, or by treatment with cyanide, etc., may be explained by either of the hypotheses put forward in (1). Moreover, the aerobic accumulation of these two products of zymase cleavage in organs (*e.g.*, germinating peas, developing buds of sycamore) in which dermal coverings may impede gaseous exchange, in injured or ageing fruits, and sometimes in other organs, may be attributed to a reduction in the activity of oxidation enzymes, without a corresponding reduction in zymase activity. The accumulation of these two substances is determined by the relative activities of the whole zymase complex (including carboxylase and alcohol-dehydrase) and of the oxidation enzymes that may attack products of zymase cleavage.

(3) Evidence has been obtained that ethyl alcohol and acetaldehyde may be aerobically consumed by tissues of some of the higher plants, provided the oxidative activity of the cells is high. Kidd and Trout supplied acetaldehyde to the orange, and, early in the storage season, to the apple ; and Kidd and West fed apple tissue with ethyl alcohol. Thomas and Foster placed apples and other plant organs under conditions that induced zymasis, and measured changes in the concentration of ethyl alcohol and acetaldehyde on returning the plant material to aerobic conditions. Foster has also fed leafy shoots, seeds, and other organs with these substances. The results of earlier work (p. 301) on the aerobic metabolism of ethyl alcohol appear to have been confirmed, since, when alcohol was consumed, the concentration of this substance fell at a slow rate. The consumption of acetaldehyde was more rapid. No convincing evidence has yet been published that the aerobic consumption of either ethyl alcohol or acetaldehyde leads to the production of carbon dioxide, or augments oxygen uptake.

(4) Klein and Pirschle fixed small amounts of acetaldehyde as acetaldomedon by supplying dimedon to tissues of the higher plants, which were respiring in air, and attributed the formation of this substance to metabolic events occurring inside the living cells. This conclusion may not have been justified (*cf.* discussion on p. 215). Accepting the evidence that zymase cleavage occurs under aerobic conditions, they inferred that this aerobic cleavage proceeds as far as acetaldehyde, and that in air this substance normally undergoes oxidation, *i.e.*, it is an intermediate product of aerobic respiration. The argument is not conclusive. It is possible that dimedon depressed oxidative activity, and in this way induced zymasis (see (2) above). These workers made no attempt to ascertain whether ethyl alcohol was produced as well as acetaldehyde.

(5) The finding of James and Norval (*156*) that carboxylase prepared from young barley tissue was active in the presence of molecular oxygen provides further evidence of the possibility that acetaldehyde may be produced by the aerobic catabolism of sugars, or of other substrates that may give rise to pyruvic acid. It is not yet known whether active oxygen (see p. 308) inhibits carboxylase action, or in some way modifies it so that acetaldehyde and ethyl alcohol are not produced in substantial amounts in cells respiring in air. If the carboxylase action of living cell is the same in air as under anaerobic conditions, the known facts may be explained in several ways. Decarboxylation of pyruvic acid could be regarded as providing some of the carbon dioxide of aerobic respiration, and the fact that acetaldehyde and ethyl alcohol do not accumulate in appreciable amounts in cells living in air could be attributed to the rapid oxidation of acetaldehyde. Of such oxidation, however, we have no evidence (see para. (3)). Alternatively it is possible that carboxylase is not called upon to operate in the decarboxylation of pyruvic acid, because in the presence

of oxygen this acid or one of its precursors is rapidly oxidized or consumed in another way. James and James (*149*) inferred from their experimental results that " pyruvic acid is continuously formed by barley tissues and is normally decarboxylated." They had detected this acid in roots and in cut darkened leaves whose carboxylase had been poisoned either with 1-naphthol-2-sulphonic acid or with acetaldehyde. Normally there is insufficient pyruvic acid present for detection by the methods used, viz., a colour reaction with ammonium nitroprusside, and the preparation of the 2 : 4-dinitrophenylhydrazone. Since pyruvic acid did not accumulate at a rate commensurate with that at which sugar is consumed in respiration, and since we do not know whether the poisons used are specific for carboxylase, and without effect on the other enzymes concerned in oxidative metabolism, the quotation given above should be considered as the statement of a hypothesis which has not been disproved, and not as that of a rigidly proved fact. A number of the findings reported in this paragraph can be accommodated within the compass of the hypothesis, but, clearly, it cannot be finally accepted until we know the aerobic fate of acetaldehyde.

(6) James and Norval (*loc. cit.*) supplied detached germinating embryos and young detached leaves of barley with M/20 pyruvic acid, and measured the resulting changes in the rate of CO_2 output, and in the respiratory quotients. The rate of CO_2 output was greatly increased, and this was attributed to the decarboxylation of the added pyruvic acid, probably as a result of the action of carboxylase. The disappearance of pyruvic acid was experimentally proved. But these workers never found free acetaldehyde in the tissues which had been fed aerobically with pyruvic acid. James and James had, however, at an earlier date reported the production of acetoin, a simple condensation product of acetaldehyde. The respiratory quotient was found to rise in a fashion " to be expected on the assumption that the pyruvic acid is being respired simultaneously with internal substances." More recently, James, Heard, and James (*155*) have stated that they regard the aerobic fate of pyruvate and acetaldehyde as an open question. The present writer is in entire agreement with them.

(7) Accepting the conclusion that in aerobic metabolism, even if acetaldehyde is formed, ethyl alcohol is not produced, we have to consider a critical question concerning the production of pyruvic acid from sugar. The oxidation of triosephosphate, one of the intermediate stages, must occur independently of the reduction of acetaldehyde to ethyl alcohol by alcohol dehydrogenase and reduced cozymase, *i.e.*, of the reaction with which it is coupled in yeast fermentation (fig. 2), and, presumably, in the higher plants placed under anaerobic conditions. For aerobic and anaerobic conditions the oxidation can be represented by the equation

$$\text{Triosephosphate} + cz = \text{phosphoglycerate} + czH_2$$

The amount of cozymase present in any cell is very small, and continuous oxidation depends, therefore, upon the rapid regeneration of cz from czH_2. Under anaerobic conditions acetaldehyde probably acts as the hydrogen acceptor in this oxidative regeneration, and ethyl alcohol accumulates as an end product (p. 60). James *et al.* (see p. 44) have obtained evidence that in barley sap the ascorbic acid system is effective in absorbing oxygen and oxidizing czH_2, and thereby in enabling the continuous oxidation of triosephosphate. The continuous aerobic production of pyruvic acid in barley seedlings, under conditions, precluding the formation of ethyl alcohol, receives, therefore, a reasonable explanation. There is no evidence yet for tissues which lack ascorbic acid oxidase.

Oxidative anabolism. In researches which have had a far-reaching influence on the interpretation of results obtained in experiments on the respiration of living cells, Meyerhof reported that in yeast and the muscle tissues of warm- and cold-blooded animals the greater part of the products of aerobic glycolysis are oxidatively reconverted into carbohydrates. Meyerhof considered that this curious cycle was dependent on the energy liberated by the aerobic oxidation of carbohydrates. When aerobic oxidation was inhibited by cutting off the oxygen supply or by hydrogen cyanide, the products of glycolysis accumulated, as the energy supply was lacking for them to be changed back into carbohydrate. Hence, on balance, considerably less respirable carbohydrate was lost by aerobic respiration than by anaerobic glycolysis or by glycolysis in the presence of cyanides. Meyerhof calculated that in muscle for every molecule of carbohydrate oxidized, five molecules of carbohydrate were virtually preserved as respirable substrate by the reconversion of lactic acid (which is the product of glycolysis in muscle) into carbohydrate. For wild yeasts, and the cultivated baker's and brewer's yeasts, he concluded that aerobic respiration protects carbohydrates from fermentation. The protection is complete in the wild yeasts, but only partial in the cultivated yeasts. It should be noted that this effect is on living yeast cells and not on zymase separated from yeast.

At about the same time F. F. Blackman (*33*) put forward a similar hypothesis in order to explain the results of Parija's experiments with apples, described in section F. It will be recalled that, in the presence of oxygen, respirable material appeared to be conserved. In the Bramley's Seedling apples, for every carbon unit lost by aerobic oxidation, 2·9 carbon units were protected from zymase cleavage to carbon dioxide and ethyl alcohol. Before Lipmann's work (see p. 308) it was generally accepted that the activity of zymase in living cells was in no way impaired by the presence of oxygen, *i.e.*, that the rate of zymase cleavage in air was not less than under anaerobic conditions; and Blackman [1] accordingly inferred that in the presence of oxygen a fraction of the labile intermediate products of zymase cleavage (*e.g.*, triosephosphate, phosphoglyceric acid, pyruvic acid, and acetaldehyde) does not undergo oxidation by oxygen respiration (*i.e.*, aerobic respiration), but is oxidatively consumed in some other way. He used the term *oxidative anabolism* to describe this second possible fate of these labile products, but did not precisely specify what this fate might be. He pictured oxidative anabolism as a process which is coupled with and dependent on oxygen respiration, and schematically represented his views as in

[1] Actually he interpreted Parija's data as showing that zymase cleavage occurred more rapidly in air. This he attributed to the more rapid activation of hexoses in the presence of oxygen. He postulated that activation is a preparatory stage to zymase cleavage (see his scheme, fig. 43).

fig. 43. Some of these ideas resemble those put forward earlier by Wortmann (see Kostytschew, *175*) and Boysen-Jensen (*46*) on the basis of the results of experiments in which they obtained I/N quotients greater than unity.

It should be carefully noted that what Parija obtained was experimental evidence that the presence of oxygen resulted in the conservation of carbon; and we have seen that there is evidence of such conservation for other tissues (section F). Blackman's hypothesis of oxidative anabolism is only one of several possible explanations of the experimental results. For example, if Lipmann's view is correct that zymase cleavage *in vivo* as well as *in vitro* is depressed by active oxygen, carbohydrates would be conserved because aerobic glycolysis would then be less active than anaerobic glycolysis. After considering the behaviour of animal tissues, Dixon and Holmes have postulated that the rate of supply of substrate to enzyme is slower in the presence of oxygen than under anaerobic conditions, *i.e.*, they explain conservation in terms of a decrease in permeability or of an increase in organization resistance. Further experimental work is required to test these different hypotheses.

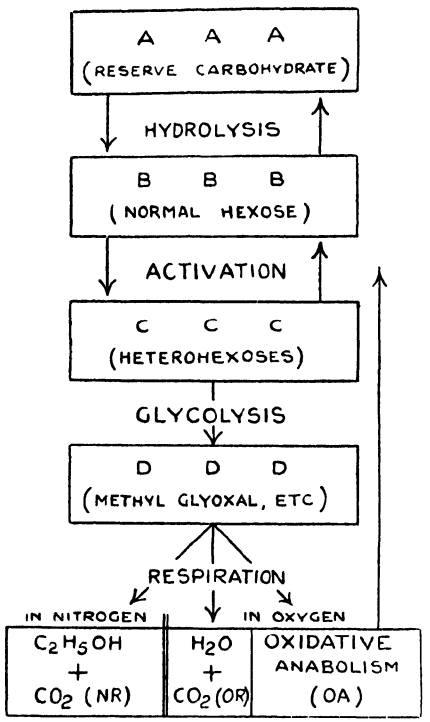


FIG. 43. Blackman's schematic representation of his views concerning the connection between the anaerobic and aerobic respiration of carbohydrates.

The problem is rendered more difficult to solve because in experiments on some organs oxygen uptake did not appear to bring about a conservation of respirable substrate (section F). Among this class are included certain germinating seeds and green leaves which are known to possess high powers of constructive metabolism, probably much higher than those possessed by fully grown fruits, in which the aerobic conservation of carbon has been proved to occur. It should be noted, however, that other germinating seeds and green leaves belong to the same class as the apple.

If there is any substance in the arguments put forward on p. 261 constructive metabolism is dependent upon the energy set free in aerobic respiration, *i.e.*, it may be described as oxidative anabolism. The fact that no evidence of the conservation of carbon has been obtained by a certain kind of experiment on a *whole* growing organ or leaf does not prove that oxidative anabolism, in the widest sense of the term, is not proceeding in some of the parts of the organ or leaf. It is true that the kind of oxidative anabolism believed by Meyerhof to occur in yeast and the muscle-tissue of animals, viz., the building back of carbohydrates from products of glycolysis, has not been proved to occur in plants, although some evidence exists that plant protoplasm has the power of converting certain substances (*e.g.*, triosephosphates, glycerol, and, possibly, C_4 and C_6 dicarboxylic acids) into carbohydrates. But there are other possible kinds of oxidative anabolism; and in recent years increasing theoretical consideration has been given to that possible form, described on p. 187, in which zymase cleavage products may serve as the source of carbon from which a large number of primary anabolites may be constructed. To prove experimentally that carbon is aerobically conserved because fats, amino acids, isoprene and benzene derivates, etc., are synthesized by oxidative anabolism at the expense of zymase cleavage products would be to achieve a major advance in definite biochemical knowledge.

On the alleged oxidative consumption of carbohydrates without zymase cleavage. We have considered on p. 299 earlier criticisms of Pfeffer's hypothesis and how these were met. Moreover, we have noted (*a*) that in developing this hypothesis Kostytschew, Blackman and others have assumed that zymase cleavage occurs in cells that are absorbing oxygen, and (*b*) that Lipmann has thrown some doubt on this crucial point. The validity of these hypotheses was also questioned when Lundsgaard reported that sodium iodoacetate completely inhibits fermentation in cultivated yeasts, but that oxygen uptake is at first hardly affected. He attributed this uptake to the respiratory oxidation of carbohydrates by the living yeast, and naturally inferred that in this organism oxidations resulting from oxygen uptake are not necessarily concerned with products of zymase cleavage. Boysen-Jensen shortly afterwards found that the fermentation of pea cotyledons soaked in a solution of iodoacetate was depressed to relatively a greater extent than the oxygen uptake, and saw in this result further evidence for the view he had long held, viz., that respiration and fermentation are independent processes. Turner demonstrated that dilute aqueous solutions of sodium mono-iodoacetate inhibit the fermentation of carrot tissue in nitrogen. The inhibitory action for a given concentration was less than that on the fermentation of yeast, because of the slow penetration of the iodoacetate into the carrot cells, particularly in the interior of the discs that he used.

More recent experiments of Lundsgaard and of others on yeast, and of Turner on carrot discs, have, however, shown that some of this residual oxygen uptake may be referred to the oxidation of ethyl alcohol that had, previous to treatment with iodoacetate, accumulated in the tissue under

experiment. Nevertheless Lundsgaard still held that the glycolysis of hexoses,—*i.e.*, the formation of triosephosphates (see p. 56)—can be completely inhibited by concentrations of iodoacetate that still permit oxygen uptake connected with the aerobic respiration of carbohydrates. Turner, on the other hand, considers that iodoacetates, by inhibiting what he describes as *triosis* (*i.e.*, the formation of triosephosphates), inhibit both fermentation and respiration, and attributes the differential effects of this inhibitor on these processes under the conditions of Lundsgaard's and his own experiments to the reduction in the presence of oxygen of the inhibitory power of iodoacetate. Turner concluded that iodoacetate has essentially the same inhibitory effect on fermentation and respiration but acts more slowly on respiration for any given external concentration. Accordingly he maintained that the results of work on iodoacetate do not in themselves necessitate the replacement of hypotheses that postulate the respiratory oxidation of zymase cleavage products by one in which respiration and fermentation are regarded as completely independent processes. Turner (*315*) has written a clear account of Lundsgaard's experiments and conclusions, and has criticized these conclusions in the light of his own experimental results.

Of evident significance to Lundsgaard's hypothesis is the discovery by Müller and others that yeasts and certain mould fungi contain oxidases that can effect the direct oxidation of certain sugars, and that these oxidases are insensitive to sodium iodoacetate. Thus in *Aspergillus niger* there is a glucose oxidase which takes part in the oxidation of glucose to gluconic acid. Further, *Saccharomyces marxianus* and *Saccharomyces exiguus* do not contain maltase, and consequently these yeasts cannot ferment maltose. But when these yeasts are grown on maltose, they absorb oxygen and give out carbon dioxide. It has therefore been inferred that they contain a maltose oxidase, which oxidizes maltose directly. It may well be that there are oxidases which play a part in producing, with the liberation of carbon dioxide, compounds containing five carbon atoms (*e.g.*, pentoses) and four carbon atoms (*e.g.*, certain vegetable acids) from hexoses. It is important to realize, however, that even if, under the agency of such oxidases, oxidation of carbohydrates in the presence or absence of iodoacetate can occur without zymase cleavage, it does not follow that under normal aerobic conditions oxidative metabolism of labile intermediate products of zymase cleavage does not take place as well. Indeed, there appears at present to be no cogent reason for abandoning the hypothesis that zymase, which may well be universally present in plant cells, in some way or other participates in aerobic oxidative metabolism.

H. Oxidizing Enzymes and Respiration

In neutral aqueous solution, carbohydrates and zymase cleavage products are not oxidized at an appreciable speed by molecular oxygen, *i.e.*, they do not act as autoxidizable substances. Hence it follows that under conditions which exist in living cells these respirable substrates lose their stability towards oxygen. Consequently the properties of the oxidizing systems that occur in living cells have aroused great interest, and much work has been done to elucidate the nature of the enzymes that can activate either molecular oxygen or oxidizable substrates, and of the substances that can act as carrier or acceptor substances.

Since oxidations other than respiratory oxidations occur in living cells (p. 267), it follows that all oxidizing enzymes are not necessarily concerned with respiration. Thus Raper has shown that when tyrosinase acts on tyrosine in the presence of air, oxygen is absorbed, and a second hydroxyl group is introduced into the tyrosine molecule (p. 191). Hydroxylation of this kind would not be a respiratory event. Consequently the enzymes concerned would not be acting as respiratory enzymes. The same remarks apply to tyrosinase when it governs oxygen uptake and pigment formation in certain developing tissues (*e.g.*, the spotted leaves of *Arum maculatum*, the black and white flowers of *Vicia faba*) and to the direct oxidases when they bring about the oxidation of phenols after injury (see p. 268). It is possible, however, that the given enzyme may function at one time in respiration and at another in some other oxidative change.

The general properties of oxidizing enzymes have already been discussed (p. 28). Here we shall consider certain recently expressed views concerning the activity of oxidizing enzymes in respiratory oxidations.

In the principal hypothesis discussed in section G, oxidation enzymes are postulated as co-operating with the zymase complex in bringing about the respiratory oxidation of carbohydrates, and in such a fashion that the respiratory quotient is unity. Although James *et al.* (see para. 5 below) have obtained evidence bearing on the problem, we do not yet know for certain at what stage of zymase cleavage oxidation enzymes become operative in diverting metabolic change from the direction of producing ethyl alcohol and the carbon dioxide of zymasis, to a metabolic sequence that leads to the absorption of oxygen and the production of carbon dioxide, but not of ethyl alcohol. Accordingly we are at present constrained to the course of merely suggesting, in the light of our knowledge of oxidation enzymes (pp. 28 to 49), some of the lines along which oxidation sequences may conceivably run.

(1) In the first place we note the probability that aerobic oxidative processes are incident on compounds containing four, three or two carbon atoms, which are either primary products or by-products of zymase cleavage (pp. 46 and 55). We may represent any one of these products as AH_2, *i.e.*, as a substance that can donate hydrogen (p. 418). Some of these compounds may be autoxidizable; others require to be activated by a dehydrase before they will part with their hydrogen to an acceptor.

(2) The oxidation systems involved may be anaerobic dehydrases or aerobic dehydrases. The former, but not the latter, act in conjunction with other oxidation enzymes, which usually, and it may be always, have a metallic component, and are sensitive to cyanides and sulphides (see pp. 35–7). Genevois (*96*) found that the respiratory quotients of many plant tissues in which carbohydrates were being oxidized rose above unity when the tissues were treated with cyanide; and Thomas and Fidler (see for example, *311*) demonstrated and measured the accumulation of ethyl

alcohol and acetaldehyde, as well as the increases in the respiratory quotient, in the tissues of certain plant organs which were respiring in air containing the vapour of hydrogen cyanide or hydrogen sulphide. These increases, like those found by James and Hora in similar experiments on barley, resulted mainly from reductions in the rates of oxygen uptake. A simple interpretation of these findings is that anaerobic dehydrase systems are involved in the aerobic oxidation of zymase cleavage products, and that the activity of one at least of the enzyme components of these complex systems is depressed by cyanides and sulphides more than is the activity of zymase. Consequently products of zymase cleavage accumulate, and more carbon dioxide is produced than can be accounted for by aerobic respiration. Further work is required to determine whether, in addition to anaerobic dehydrases, aerobic dehydrases and other systems insensitive to cyanide are concerned with respiratory oxidations in the higher plants (*cf.* the note on the respiration of yeast, p. 34). An important point concerning technique to make in passing is that it has not yet proved an easy matter to extract active dehydrase preparations from tissues of the higher plants.

(3) Among the hydrogen acceptors that may occur in plants are oxygen, codehydrases I and II, flavoprotein, cytochrome, dehydroascorbic acid, orthoquinone, certain vegetable acids, and glutathione. AH_2, when activated by a specific dehydrase, may donate its hydrogen to one of these acceptors, which will thereby be reduced. Since the concentration of organic acceptors in living cells is usually extremely low, very little AH_2 will be oxidized to A by this initial dehydrogenation. For the continuous oxidation of AH_2 it is clearly essential that such acceptors should be regenerated. This all-important regeneration takes place as a result of a succession of oxidations. We have already discussed possible sequences in the dehydrogenation of dihydrocodehydrases I and II, dihydroflavoprotein, reduced cytochrome, ascorbic acid, dihydroxyphenols, succinic acid, and reduced glutathione. We recall that James *et al.* (see p. 306) place codehydrase I (*cz*) in a key position as a hydrogen acceptor in the determination of metabolic events during anaerobic and aerobic glycolysis in barley, and that they associate the aerobic regeneration of *cz* from czH_2 with an oxygen uptake.

(4) Under aerobic conditions, in the final dehydrogenation, both in anaerobic and aerobic dehydrase systems, oxygen acts as the hydrogen acceptor. It has been widely held that hydrogen peroxide is produced; but this substance has never been detected in plants. Catalase is universally present in the cells of aerobes, and would decompose the peroxide, yielding water and oxygen.

(5) The main function of oxygen appears therefore to be that of a hydrogen acceptor in aerobic and anaerobic dehydrase systems. In the latter it is eventually responsible for the continuous regeneration of all the hydrogen acceptors involved in a particular oxidation. Among the special enzymes concerned with the action of oxygen as a hydrogen acceptor in anaerobic dehydrase systems, are cytochrome oxidase, catechol oxidase and other direct oxidases of the higher plants, and ascorbic oxidase. The action of these enzymes is inhibited by cyanides. A cytochrome oxidase has been detected by Hill and Bhagvat (see p. 37) in the cotyledons of Phaseolus, but we cannot yet state that this type of enzyme is universally present in the tissues of the higher plants. Boswell (*42*) attributes a considerable fraction of the respiratory oxygen uptake of potato tissue to the activity of a catechol oxidase, but Onslow (*214*) maintained that direct oxidases only occur in about sixty per cent. of the higher plants. Ascorbic

oxidase may be present in still fewer. James *et al.* (see pp. 44 and 306) have obtained evidence that in barley ascorbic oxidase forms part of a system which produces pyruvic acid by the aerobic oxidation either (*a*) of lactic acid, or (*b*) of triosephosphate by way of phosphoglycerate. They point out that the evidence for the occurrence of reaction (*b*) is the first to be obtained for the higher plants of a linkage of a generally occurring event in glycolysis with a "well-investigated oxidation system" which is "typical of plants".

(6) The part played in respiration by peroxidases, which are present in all plant cells, is still obscure. Like other hæmochromogen catalysts they are sensitive to cyanides and sulphides, but this does not prove that they function in respiratory processes. The idea that peroxidase systems may link with other enzyme systems (pp. 30 and 34) is suggestive.

(7) It is not yet known at what stage or stages carbon dioxide is split off in the aerobic respiration of carbohydrates. We have already seen that oxidation enzymes form part of the zymase complex, and that acids such as pyruvic acid are produced from sugars even in the absence of oxygen. As we shall point out in the next chapter it is probable that other α-ketonic acids are produced as a result of dehydrogenations and hydrolytic oxidation reductions under aerobic conditions; and it is known that such ketonic acids are decarboxylated by the enzyme carboxylase. The only other plant enzymes which have been shown to decompose their substrates *in vitro* with the production of carbon dioxide are formic dehydrase and urease. The functions of these enzymes *in vivo* are not known.

From the foregoing discussion it is clear that, as a result of their analytical treatment of the subject of respiration, biochemists now picture that highly elaborate enzymic mechanisms are operative at the respiratory centres of protoplasm. Analysis is not yet complete; but it may well be that several distinct enzymes, with associated coenzymes and necessary ions (*e.g.*, phosphate and magnesium ions), may be concerned as an organized protoplasmic system in the oxidation of sugars into carbon dioxide and water, by way of zymase cleavage products, and thereby liberating energy that is harnessed in the service of the organism. Little is known about other possible types of oxidation; but it is clear that there must be qualitative differences in the composition of such centres as bring about the oxidation of sugars to compounds containing five carbon atoms, and of centres at which substrates such as amino acids and fatty acids may undergo respiratory oxidation. In all instances the food which is consumed, and, therefore, the oxidizable substance (AH_2) formed from it, will be in continuous supply, and the final hydrogen acceptor, viz., atmospheric oxygen, in unlimited supply.

CHAPTER XV

THE METABOLISM OF DI- AND TRICARBOXYLIC ACIDS

A. Introduction

THE latin word *acidus* means sour. It was doubtless invented to describe the taste of certain plant organs. We now know that such sourness, which is not to be confused with the astringency of tannins, is largely owing to the production and accumulation of di- and tricarboxylic acids (p. 424).

These acids occur in the free state, and as acid or normal salts. They may have several functions. Evidence grows that many of them are produced in all plants, and are intermediate metabolites in a variety of anabolic and catabolic processes, and not merely end products of metabolism, which may possess, at times, secondary biological significance. Calcium oxalate is probably a waste product. Being insoluble, it is produced in the cells in which it accumulates, and there is no certain evidence that once formed it is ever consumed. The crystals may, at times, have a protective function (p. 180). In Thoday's department at Bangor a close study has been made of the acid metabolism of the genus Kleinia, and much attention has been given to the accumulation and distribution of calcium oxalate and calcium malate in the tissues. Jackson and Thoday (*147*) found that, even after prolonged starvation in the dark, calcium malate did not disappear in the neighbourhood of sprouting shoots or roots. They concluded that calcium malate is not a food reserve in Kleinia.

We gave on pp. 44 to 48, and we shall give in this chapter, chief consideration to problems of production and consumption, and not to the possible catalytic or other functions of such acids as have accumulated.

B. The Measurement of Acidity

The recognition and estimation of each individual acid are the objectives of the analyst. Franzen and others developed qualitative methods of analysis which have been described and used by Nelson (*207*). In the 1930's quantitative methods have been greatly improved. Vickery and Pucher (*317*) consider that " The day is now past when the results of a simple titration may be calculated in terms of malic or citric acid and interpreted in any meaningful way," and cite papers where descriptions may be found of specific methods of analysis for all the acids mentioned in this chapter.

Total acidity as the sum of titratable and non-titratable acid. Nevertheless simple titration may continue to give suggestive

preliminary information. Substances with replaceable acid hydrogen may be extracted from minced tissue by boiling with water. The solution is then titrated with standard alkali. Phenolphthalein has often been used as the indicator. Contributions to the titratable acidity so measured would, of course, be made by all the acid salts as well as by the free acids present.

To evaluate the total accumulation of products of acid synthesis one must determine the hydrogen equivalent of all the organic anions that are present in the tissue analysed. A fraction of these anions will be combined in normal salts of metals and ammonium, or with organic bases (*e.g.*, alkaloids). Although total acidity is the only exact measure of the total acid anion accumulation in a plant, titratable acid may often be used as its measure in short duration experiments, because, in these, acid is produced or consumed much more rapidly than it enters into combination (Bennet-Clark, *21*).

Sporadic bursts of ammonia production, by neutralizing some acid, may occasionally cause a quick increase in non-titratable acidity; but, as a rule, removal by neutralization is quantitatively significant only when seasonal drifts are being studied. The anions of nitrates, sulphates, and phosphates, absorbed from the soil are gradually used in anabolism. The residual cations combine with such titratable acids as may be present in the sap to form soluble (*e.g.*, potassium malate) and insoluble salts (*e.g.*, calcium oxalate). It has been shown that old plants of the Crassulaceae and Cactaceae have less titratable and more non-titratable acid than young ones. Bennet-Clark has stated that the concentration of free malic acid in plant tissues rarely exceeds 0·05 per cent. of the fresh weight and is usually much lower, though the total malate content in acid plants may be as high as two per cent. of the fresh weight.

Bennet-Clark and Woodruff (*27*) have described a method for precipitating acid anions as insoluble salts of lead, which are then decomposed with sulphuretted hydrogen. The acids set free are titrated with standard alkali. The total acidity so measured includes malate, citrate, succinate, oxalate, sulphate and phosphate.

Hydrogen ion concentration. (i.) *Acid metabolism and pH values.* If there are no free cations present in cell sap, titratable acid will accumulate when the rate of production exceeds that of its consumption. The *pH* value will tend to fall, but not to a very low value because vegetable acids are weak acids and because of the presence of buffers (p. 461). Pucher *et al.* (*239*) found that the *pH* of the sap from rhubarb leaves declined from 4·73 to 4·10 from April to August. During this period acid accumulation, which is not all titratable acid, is vigorous (see fig. 49). Evidently, parallel processes which set free cations, by causing the formation of acid or neutral soluble salts, or the precipitation of insoluble salts, would oppose the falling tendency.

In many succulents and the Polygonales, only slight decreases in *pH* occur while total acidity progressively rises to reach high values.

Conversely, *pH* will tend to rise when the rate of consumption of titratable acid exceeds that of its production. This happens in some fleshy fruits (*e.g.*, ripening oranges, see p. 320).

(ii.) *Some recorded pH values.* Even the relatively alkaline plant saps are acid to litmus. Chibnall (59) has reported relatively high *pH* values for the sap of certain leaves, *e.g.*, fig (6·8), spinach (6·5), scarlet runner (6·0), cabbage (5·6); but, in general, the *pH* values of plant saps are less than 5·5. We cite as examples a few of the recorded values for plants whose acid metabolism has been much studied: leaves of many succulents and members of the Polygonales (4 to 5); rhubarb rhizomes (5·56), buds (5·22), petioles (3·26 to 3·67), leaves (4·73 to 4·10); Valencia oranges (immature 2·88, mature 3·78); leaves of Pelargonium (2·82), Begonia (1·53), and Oxalis (1·5). These values are, of course, not constants. They vary from cell to cell in a given tissue, and from time to time. The *pH* of cell sap is not determined by that of the soil solution: it is a genotypic character.

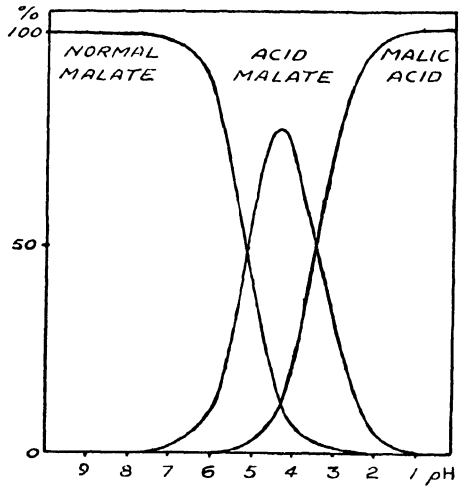


FIG. 44. The percentage of total malate present as normal malate (M″), acid malate (HM′) and malic acid (H_2M) in solutions of various *pH*. (From Bennet-Clark.)

The *pH* of a decinormal solution of hydrochloric acid, which is a strong acid, is 1·07 (see p. 463). In some plant organs (*e.g.*, orange fruits), the concentration of titratable acid may approach normal. Evidently the acids produced are weak acids, and their weak ionization may be depressed to some extent by buffers. Oxalic is stronger than the other dicarboxylic acids or the members of the citric acid group. This fact may account for the lower *pH* values found for Begonia and Oxalis.

(iii.) *pH as an index of the states of organic anions in solution.* Bennet-Clark (*21*) has discussed the various equilibria which may co-exist in a solution containing the acid salts of a dibasic acid, when acid or alkali is added to it. The organic anions set free in metabolism may remain within the sphere of influence of hydrogen, either in

molecules of undissociated or as anions of electrolytically dissociated acid; or they may become linked with cations such as potassium or ammonium, and so form acid or normal salts. The *pH* of the sap gives an index of the relative amounts of organic anions that are in these different states (fig. 44). For example, if, after a period of malic acid accumulation, the *pH* of cell sap is about 5, as frequently happens (see para. (ii.)), we may infer from the graphs that about three per cent. of the malic acid will be present as free acid, about fifty-one per cent. as acid malate, and about forty-six per cent. as normal malate. If the corresponding citric acid curves are not very different, we could conclude that in immature oranges most of the acid present is in titratable forms.

C. Notes on the Occurrence of the Individual Acids

The reader desiring further information about the distribution of vegetable acids will find it, and, also, extensive bibliographies, in the books or papers of Pfeffer (*228*), Czapek (*67*), Bennet-Clark (*21*), and Nelson (*207*).

Oxalic acid ($H_2C_2O_4$). Scheele discovered oxalic acid in Oxalis and Rumex, and found the calcium salt in rhubarb. Crystals and crystal bundles (raphides) made of this salt dissolve in hydrochloric acid, but, unlike those of calcium carbonate, not in acetic acid. Soluble oxalates may be identified by the precipitation which occurs on adding an acetic acid solution containing calcium ions. These simple tests have been used to explore the plant kingdom for the occurrence of oxalic acid or oxalates.

The searchers found the oxalate ion seldom in the red and brown algæ, but frequently in the other Thallophyta and the higher plants. Among the Angiosperms, Oxalis and Pelargonium belonging to the Geraniales, Begonia to the Parietales, Salicornia (*e.g.*, of a succulent halophyte) and Mesembryanthemum (*e.g.*, of a desert succulent) to the Centrospermae, and Rumex and Rheum to the Polygonales, contain tissues which, at some stage in the lives of the plants, may be rich in oxalate.

Free titratable acid may accumulate in high concentration. Low *pH* values (*e.g.*, 1·5 to 2·0 for Begonia sap) show that the oxalic acid must be present mainly in the free state. On the other hand, oxalate plants in the Centrospermae have nearly a neutral reaction. They contain very little titratable acid. A high proportion of their dry matter is calcium oxalate. In some Cactaceae also, crystals of this salt may account for fifty to eighty per cent. of the dry weight.

Malic ($C_4H_6O_5$), oxalacetic ($C_4H_4O_5$), succinic ($C_4H_6O_4$), and fumaric ($C_4H_4O_4$) acids. Scheele obtained malic acid from fruits of barberry, elder and cherry. We now know that *l*-malic acid is probably always present in fleshy fruits (especially unripe ones), sometimes predominating over other acids (*e.g.*, in sour apples, mountain ash berries) but often occurring in less amounts than other acids (*e.g.*, than citric acid in lemons, than tartaric acid in grapes).

In 1800 Vauquelin found calcium malate in Sempervivum and in genera belonging to the Crassulaceae, and it was later reported that malic acid or malates (see p. 320) are widely distributed among the flowering plants, and are present in some cryptogams. Czapek states that malic acid is not less frequently produced than oxalic acid, but is not so easily detected. Bennet-

Clark gives a list of families (*e.g.*, Polygonales, Rosales, Opuntiales) in which there are noteworthy accumulations of malic acid. Some of these families are widely separated phylogenetically. In some genera of the Crassulaceae soluble malate may account for more than half the dry weight. In shoot systems the acid is found mainly in the lævo form, but the presence (*e.g.*, in the rhizome of rhubarb) of the inactive acid, *i.e.*, equal quantities of *d*- and *l*-malic acid, has been reported.

At least traces of the related acids succinic, fumaric, dioxymaleic, and of citric and oxalic acids are usually found with malic acid. Succinic and fumaric acids may attain high concentrations, but do so less frequently than citric, malic and oxalic acids. In Lactuca, succinic was found with malic acid in considerable and approximately equal amounts. Fumaric acid accumulates in certain genera of the Papaveraceae (*e.g.*, Fumaria, Corydalis). In the analytical records there is often a substantial "unidentified acid" fraction, clearly showing that much remains to be discovered about acid metabolism. Monobasic acids (*e.g.*, acetic, glycollic, lactic and gluconic acids), and malonic and glutaric acids may be among the acids occurring in this fraction of the acids named in fig. 49; oxalacetic acid and the other ketoacids are probably too unstable to accumulate.

Tartaric acid ($C_4H_6O_6$). In 1769 Scheele obtained *d*-tartaric acid from the hard brown crust, tartar (potassium hydrogen tartrate), which separates in the vats during the fermentation of grape juice. In addition, racemic acid (*d*-*l*-tartaric acid) has been found in grapes. In 1848, Pasteur performed his famous resolution of this inactive acid into its optical isomers.

Tartaric acid accumulates in relatively high concentrations in some organs (*e.g.*, fruits and leaves of Vitis). Low concentrations have been found in fungi, lichens, Lycopodium, senna leaves, some leguminous fruits, etc.: indeed, like other members of the malic acid group, it is widely distributed. Some of the other vegetable acids (*e.g.*, citric acid in grapes) are always present with it. As a rule it contributes little to the total acidity of fleshy fruits. In these organs malic and citric acids are usually the predominant acids.

Citric acid ($C_6H_8O_7$). Scheele separated citric acid from Citrus fruits. In one modern analysis over ninety-eight per cent. of the total acid in a grape fruit was found to be citric acid, the rest being malic (one per cent.), tartaric (0·05 per cent.) and oxalic (0·23 per cent.) acids. In another, 100 gm. fresh weight lemon gave 0·92 gm. citric and 0·18 gm. malic acid. As was pointed out above, it is present in most fleshy fruits.

It also accumulates in the free state or as salts in other organs (*e.g.*, the leaves and rhizomes of rhubarb). Many mould fungi produce it. On a large scale it is prepared by fermenting sugars with Citromyces.

The isomeric isocitric acid is the chief acid of blackberry leaves and fruits. The allied acids, tricarballylic ($C_6H_8O_6$) and aconitic ($C_6H_6O_6$) have been detected in unripe beet.

D. The Acid Metabolism of Fleshy Fruits

The accumulation of acids. In those growing and unripe fruits which have a sour taste, vegetable acids accumulate either because they are translocated from the leaves to the fruits or because fruit tissues, in growing, synthesize the acids from sugars and other substrates migrating thereto. Although malic acid had been detected in moving sap, the second explanation was favoured by Czapek (*67*); but, in the light of Allsopp's research (p. 332), the

first explanation may have to be reconsidered. We recall (see section C) the reports that grape leaves and fruits contain tartaric acid, and blackberry leaves and fruits isocitric acid. One possible interpretation is that these acids are translocated from leaves to fruits in these plants. Inasmuch, however, as different tissues in the same shoot may possess similar metabolic powers, the leaves and fruits of a given species may be able to produce the same acid from sugar.

Czapek summarized the earlier work on this subject, and also considered the biochemical meaning of the substitution during ripening of a sweet for a sour taste. He pictured a dynamic system, and attributed both the accumulation and disappearance of acids in attached or detached fruits to differences in the relative rates of the metabolic processes concerned in production and consumption. The gradual increase and decrease in the concentration of malic acid in an acid apple is graphically represented in fig. 24. Bennet-Clark has expressed the opinion that the acid metabolism of senescent fruits may not be very different from that of succulent shoots, starving and turning yellow in the dark. Czapek virtually suggested that all the enzymes concerned with acid metabolism may be present from the beginning of ovarial or receptacular proliferation, and may retain some activity until the cells grow very old. In that event acids would be produced and consumed throughout a fruit's life. In the young fruit acids would accumulate because production is faster than consumption. Czapek thought that, as a fruit ages, the rates of acid production and consumption both decline, but the former does so more rapidly than the latter. Eventually consumption would become faster than production, and, if the volume of the fruit decreases relatively more slowly, the acid concentration would get less. Certainly, the absolute amount of acid in a single fruit would decline.

The methods of production (*e.g.*, from amino acids, or by the union of carbon dioxide and pyruvic acid, arising by glycolysis) and consumption (*e.g.*, by conversion into amino acids or carbohydrate, by complete oxidation, or by the gradual formation of salts) that are discussed in sections E and F may be the same in fleshy fruits as in other organs. Because of the large amounts of carbohydrate present as polysaccharides in unripe and as sugars in ripe fruits, it is difficult to prove by quantitative experiments whether acid production can be attributed to a fraction of the carbohydrate lost, or whether, in the consumption of acids, some is converted into carbohydrate or into respiratory carbon dioxide. Enlightening evidence may come from investigations, such as those outlined in section E, on enzymes which govern the acid metabolism of developing and maturing fruits.

The consumption of citric acid in ripening oranges. Doubtless the time will come when the production and consumption in fruits of all the acids mentioned in section C will require separate treatment. The present subsection is written because Sinclair *et al.* (*267*) have recently described an experiment of a superior kind. They analysed the organic acid of the juice from immature green and from ripe oranges. In table XVI we record the *concentrations* of citric acid and of other acids, which they grouped together as malic acid. It is presumed that the volumes of the fruits did not change much, and that the changes in concentration, therefore, reflect changes in the absolute amounts of each fraction analysed.

TABLE XVI. *Mgs. titratable plus combined acids in a ml. of orange juice*

	Immature green	Mature
Titratable citric acid .	25·5	9·3
Titratable malic acid .	1·6	1·4
Combined acid . .	2·8	2·9
pH	2·88	3·78

These investigators showed that, in spite of buffering, the *pH* of orange juice is only a little higher than that of pure citric acid of the same concentration. It is obvious that free citric acid was consumed, and that this accounts for the rise in *pH*. The products of consumption are neither other acids nor salts. There are suggestions in fig. 1 about reactions by which citric acid might be continuously converted into respiratory carbon dioxide and water or into sugars, but experimental evidence for the higher plants is very meagre.

E. The Metabolism of Di- and Tricarboxylic Acids by Organs other than Fruits

In 1804 de Saussure reported that, even in the absence of an external supply of carbon dioxide, illuminated branches of Opuntia can produce oxygen. He suggested that the plant itself, by consuming its own substance, gives off carbon dioxide, which is then used in carbon assimilation. Shortly afterwards, calcium malate was detected in Sempervivum and other Crassulacean genera, and it was noticed later that some of these genera (*e.g.*, Bryophyllum) may, in the morning, have a strongly acid taste, which gets less during the day. A comparison was made of this diurnal phenomenon with the seasonal production and disappearance of acids in such fruits as have a sour taste when they are growing and a sweet one when ripe. Many

further experiments were made in the last century on acid metabolism and the gaseous exchanges connected therewith (see Czapek, *67*). Bennet-Clark (*21*) took also into account more recent work in his critical review, and discussed some possible biochemical mechanisms, which might control the production and consumption of acids. We shall base our treatment of the subject on his articles, but shall also use knowledge that has been gained since they were written.

Diurnal changes in titratable acidity. Such changes occur in Oxalis, Vitis, Rumex and other non-succulent plants, as well as in

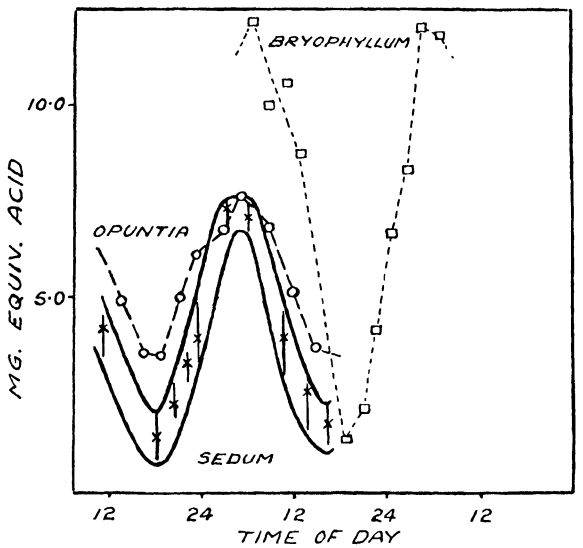


FIG. 45. Diurnal changes in the titratable acidity of 100 gm. fresh weight of Sedum and Bryophyllum leaves, and Opuntia joints.[1] (From Bennet-Clark, *21*.)

succulent plants belonging to the Crassulaceae, Cactaceae, and other natural orders. The highest recorded percentage increase in the night is 1,260 per cent. for Bryophyllum. In the meadow geranium, a non-succulent plant, an increase of 700 per cent. has been found. The published values are often much less than thirty per cent., and there are some negative values in the list Bennet-Clark gives. The values tend to be low in young leaves and, up to a certain stage of development, to increase as the leaves grow older. Since climate and soil conditions also may affect acid metabolism it is not

[1] The broad bands in figs. 45, 46 and 47 give an idea of the sampling errors in the measurements. Clearly the major fluctuations can be accepted as being significant without detailed statistical analysis.

surprising that different workers have reported different values for a single species. For example, Thoday and Evans found an increase of 440 per cent. in *Kleinia articulata*, a succulent plant belonging to the Compositae; other records for this species are zero and thirty per cent.

In the Crassulaceae it is malic acid which is produced and consumed. In *Kleinia articulata* malic acid, produced in the outer cortex, which contains chlorenchyma, is chiefly responsible for the acid periodicity (see Thoday and Jones, *301*). Presumably also in the Cactaceae the amount of malic acid present fluctuates diurnally. Genera in

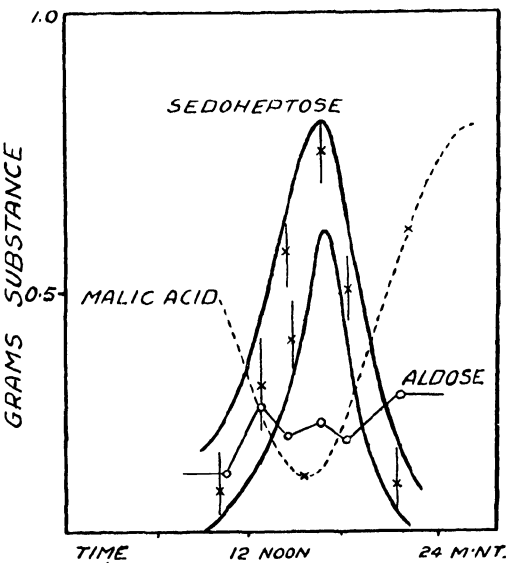


FIG. 46. Diurnal fluctuations in acid, aldose and sedoheptose content of 100 gm. fresh weight of detached leaves of *Sedum præaltum* in late June. (From Bennet-Clark, *21*.)

this family may be rich in oxalate, but we have no definite information about the production and consumption of this acid.

The relation of acid production to sugars previously photosynthesized. In the Crassulacean type of metabolism titratable malic acid decreases in the day and increases at night (see fig. 45), and greater amounts accumulate when the conditions on the previous day have favoured photosynthesis. These amounts may be relatively great after a warm sunny day, and relatively small if green shoots are illuminated in an atmosphere free from carbon dioxide. The diurnal fluctuations are confined to green parts of plants. There exist reports of acid concentrations in the green parts of variegated

leaves five times greater than those in the white parts. All these facts obviously point to a possible connection between acid production and previous photosynthetic activity.

This conclusion is supported by the results of quantitative analysis. Bennet-Clark showed that in Sedum there is an unusual sugar known as sedoheptose ($C_7H_{14}O_7$). Over a fourteen-hour period its concentration varied inversely with that of malic acid (fig. 46). Here we note especially the loss in sedoheptose at night while malic acid was accumulating. The aldose concentration did not change much. He also confirmed Wolf's finding at Leipzig that in Bryophyllum loss of sugar in the dark is more than sufficient to account for all the malic acid produced. Although it was held at Leipzig that in rhubarb and Begonia vegetable acids arise from the deamination of amino acids, it was clear to Wetzel and Ruhland by 1931 that in the Crassulaceae malic acid is produced by carbohydrate catabolism.

The occurrence of normal sugar respiration during acidification. While the acid concentration increases in the dark (fig. 45) the measured respiratory quotients may fall to very low values. Indeed, they sometimes approach zero. A possible explanation is that malic acid is produced by the partial oxidation of sugar.[1] There is no objection to the arithmetic of the equation

$$2C_6H_{12}O_6 + 3O_2 = 3C_4H_6O_5 + 3H_2O,$$

which implies that oxygen absorption without CO_2 production accompanies the formation of acid. If this equation is correct, and since some carbon dioxide is usually given off during acidification (*i.e.*, the respiratory quotient is never actually zero), normal respiratory oxidation may accompany acid formation as a parallel or even a coupled process. The value of an observed R.Q. would then be determined by the relative rates of acid formation and of the complete oxidation of sugar.

It has long been known that during a prolonged sojourn in the dark the R.Q. may rise and approach unity. Bennet-Clark's experiments (fig. 47) indicate that sugar respiration goes on in the dark continuously, side by side with acid metabolism, whether acid is being produced or consumed, and does not replace it. As Bennet-Clark pointed out, a noteworthy point in the charts is that the maxima for acid production nearly coincide with the minima for CO_2 output. A similar inverse relation was also noted by workers at Leipzig.

The consumption of acid during prolonged starvation in the dark. Bennet-Clark proved that in the dark the amount of malic acid present in detached Sedum leaves, after rising steeply, declines

[1] This explanation was in favour for many years. The present writer's alternative explanation, based on a hypothesis put forward on p. 327, is given on p. 268.

gradually (fig. 47), but may show two or more resurgences during the period of starvation. (Only one is shown on the chart in fig. 47.) He obtained similar results with Crassula. All succulents do not always behave in the same way. Thoday and Richards (*302*) found in Kleinia a rise in malate during the first night, but stated that the high concentration reached fell only after prolonged starvation. The nineteenth-century workers reported the deacidification of tissues in the dark when the temperature was raised from 15° C. to 45° C.,

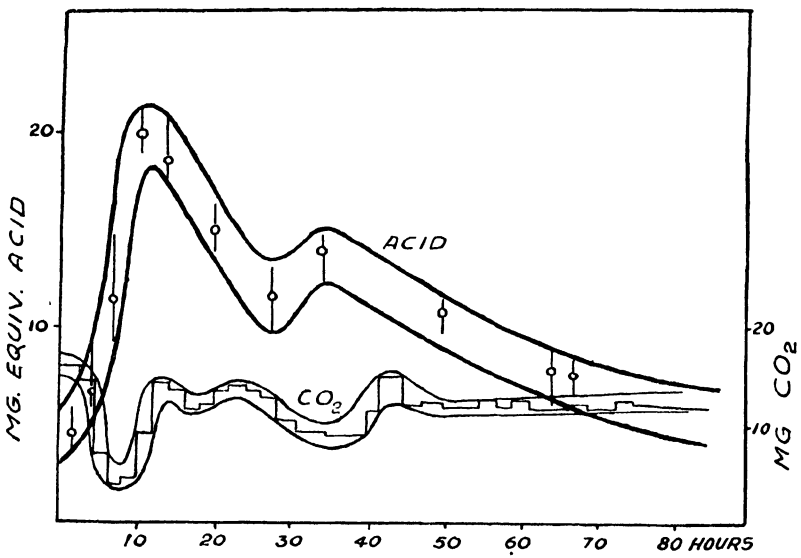


FIG. 47. Fluctuations in the acid content and in the CO_2 output per hour of 100 gm. fresh weight of detached leaves of *Sedum prœaltum* kept at 21° C. in continuous darkness from 8 p.m. on June 17th. (From Bennet-Clark, *21*.)

and pointed out that it was accompanied by the giving off, not of oxygen, but of carbon dioxide.

In analysing the results recorded in fig. 47 Bennet-Clark stressed the fact that when malic acid was rapidly consumed it disappeared at rates greater than could be accounted for by the rate of CO_2 output. Conceivably some of the malic acid might have been oxidized to carbon dioxide,

$$C_4H_6O_5 + 3O_2 = 4CO_2 + 3H_2O$$

but much of it must have given rise to something else. Inasmuch as accumulation of ammonium salts, amides and esters only accounted for a small fraction, Bennet-Clark inferred as the only alternative then conceivable that malic acid is consumed by oxidative anabolism

with the production of carbohydrate. He adduced support for this inference from the high values, often greater than two, obtained by himself and others for the respiratory quotient when acid is disappearing in the dark. For the complete oxidation of malic acid according to the above equation the quotient would be 1·33, which would mean a measured quotient less than this value if sugar were simultaneously oxidized. Bennet-Clark's empirical equation for the anabolism of citric acid by a mould fungus (p. 336) indicates how atmospheric oxygen may be spared, and, in consequence, the measured R.Q. may be much greater than unity, although actually, sugar alone is undergoing respiratory oxidation. A parallel equation for the anabolism of malic acid is given below.

Photodeacidification. It should be noted that the decrease in titratable acidity of green shoots in the dark is very slow in comparison with the rate of photodeacidification. This does not necessarily mean that the biochemical mechanism of acid consumption is different, or that the rate of consumption decreases. The governing cause may be a greater rate of production in the dark.

The only known way by which titratable acid might quickly be neutralized is by the production of ammonia in protein catabolism. There is never sufficient produced to account for photodeacidification.

Any explanation of this process must account for de Saussure's observation, confirmed beyond doubt by Mayer, that oxygen production accompanies it; and for the later finding that the photosynthetic quotient, *i.e.*, *the volume of CO_2 absorbed from the outside air/the volume of oxygen liberated from the plant's outer surface*, which is unity for normal photosynthesis, may be much less than unity when malic acid is disappearing in the light. Spoehr (*280*) has reported values less than 0·5. When oxygen is produced, as it can be, in the complete absence of external carbon dioxide, the quotient is zero. In ordinary air normal photosynthesis accompanies any modified form related to the disappearance of acid, and carbohydrates accumulate. In illuminated leaves of Sedum (fig. 46) Bennet-Clark found big increases in the concentration of sedoheptose. The aldose concentration did not change much: there was no cane sugar present. We shall assume that hexoses are intermediates in the production of sedoheptose.

One possibility is that malic acid undergoes anabolism.

$$3C_4H_6O_5 + 3H_2O = 2C_6H_{12}O_6 + 3O_2.$$

The production of oxygen without the absorption of CO_2 would lower the measured photosynthetic quotient. Alternatively malic acid is completely oxidized,

$$C_4H_6O_5 + 3O_2 = 4CO_2 + 3H_2O,$$

and the carbon dioxide produced is immediately used in photo-

synthesis, thus sparing atmospheric carbon dioxide. The measured photosynthetic quotient would then be less than unity ; how much less would depend upon the relative rates at which CO_2 coming from the outside air and that produced by the oxidation of acid arrive at the surfaces of chloroplasts.

Hypotheses concerning the metabolic nature of photodeacidification and dark deacidification. (i.) *Metabolism as the cause of photodeacidification.* It was known in the last century that oxalic acid can undergo photo-oxidation in the presence of ferric chloride. More recently, Spoehr (*280*), on the basis of his own experimental results, attributed photodeacidification to a purely chemical photolytic decomposition of malic and other acids. Czapek cited evidence that acids do not disappear quickly when killed or narcotized tissues are illuminated ; and Bennet-Clark, for the same reason and because the temperature coefficient is greater than two, also advocated the view that photodeacidification is a metabolic and not a photochemical act.

The mass of evidence, old and new, strongly supports the conclusion that there is a continuous metabolic production and consumption of organic acids, and that the relative rates of these processes vary with the external conditions, particularly with those governing the rate of photosynthesis and respiration. If the rate of production is greater than that of consumption the acids accumulate, but they tend to disappear if the relative rates are reversed. Physiological investigations have caused biochemical problems to be defined. For example, we are now reasonably certain that acids can be produced from carbohydrates : it is the biochemist's task to determine the stages by which they are formed. Some progress has already been made (see pp. 44 to 48). The description will be complete when, for each acid, the separate stages have been carried out *in vitro* under the agency of their specific enzymes. We have little definite knowledge about the metabolism which causes acids to disappear. We shall consider again Bennet-Clark's hypothesis that they can be converted into carbohydrate. The biochemist has another task to perform in testing this hypothesis and others concerning the consumption of acids. Until the biochemical mechanism of the production and consumption of acids has been elucidated with success approaching that achieved in the analysis of alcoholic fermentation (see p. 334), it is clear that overall equations of the kind given in this chapter merely summarize informed speculations.

(ii.) *Wolf's hypothesis of the qualitative control of acid metabolism by the CO_2 concentration contained in intercellular spaces.* Wolf emphasized the fact that there is one critical difference between the conditions in which green shoots undergo photodeacidification and those in which malic acid accumulates in them in the dark; the concentra-

tion of CO_2 in the intercellular spaces will usually be greater and that of oxygen less in the dark than in the light. Indeed, he thought that, in the dark, the CO_2 concentration might become so high and that of oxygen so low that the metabolic disturbance called CO_2 zymasis (see p. 303) might set in. He gave a biochemical explanation why malic acid might then accumulate. Bennet-Clark (*21*) looked for evidence of zymasic metabolism in shoots that were producing malic acid, but found little or none. Nor has the present writer, in extensive investigations on many tissues, ever encountered CO_2 zymasis, with high acetaldehyde accumulation, as a phenomenon occurring under natural conditions. Bennet-Clark also pointed out that the accumulation of malic acid cannot be attributed to oxygen want, since the rate of accumulation is increased by enriching the external atmosphere in oxygen. Wolf's hypothesis need not detain us further, but its starting point should be remembered, viz., that metabolic events may be controlled by the gas composition of the internal atmosphere, and that this will be determined by the relative rates of photosynthesis and respiration.

(iii.) *An alternative hypothesis to Wolf's; CO_2 as a metabolite; the quantitative control of acid metabolism by the CO_2 concentration in the interior.* The present writer considers that the speculation summarized by the overall equation written below has some attractions:

$$C_6H_{12}O_6 + 2CO_2 = 2C_4H_6O_5.$$

Accepting Bennet-Clark's view that malic acid is consumed by being synthesized into sugar, we might be justified in putting the sign for a reversible reaction in the equation, because Bennet-Clark has allowed that, under certain conditions (see p. 329), it is possible, but not probable, he thinks, that up to a third of the malic acid which disappears may be converted into carbon dioxide. The equation disregards any reaction involving oxygen uptake with which malic acid production may be necessarily coupled.

It accounts for the striking fact that malic acid *accumulates* in green succulents only when there is a considerable supply of CO_2 available in the interior, *i.e.*, only in the dark and not in the light. It does not exclude the possibility that malic acid is *produced* even in the light at the expense of sugar and of such CO_2, liberated in normal respiration, as is not used in photosynthesis. There is no evidence that the enzymes that control acid formation lose their activity in the light. Evidently the R.Q. would approach zero if, during active formation of acid in the dark, so much CO_2 was consumed by the cells that very little escaped into the outside air.[1] One possible explanation of the troughs in the CO_2 output curve in

[1] See p. 268 for the present writer's interpretation of low R.Q.'s which have been recorded since de Saussure's day for succulent and other plants showing acid metabolism.

fig. 47, and of the similar troughs Thoday and Richards (*302*) found in the respiration curves of starving Kleinia, is that they correspond to active periods of CO_2 assimilation in the production of acids in the dark. Bennet-Clark has pointed out that the peaks in the acid production curve in fig. 47 coincide nearly, but not exactly, with the troughs in the CO_2 output curve. We recall (see p. 323) that similar observations were made at Leipzig.

The fact, which has been recorded in the older literature, that photodeacidification is retarded in the presence of high concentrations of CO_2 is no longer acceptably explained by Wolf's hypothesis. It might be attributed to the tendency for malic acid to accumulate under all conditions, in the light as well as in the dark, when a sufficient supply of CO_2 is assured.

The results of preliminary experiments performed in the summer of 1946 at Newcastle by Beevers have been reported at a meeting of the Society of Experimental Biology. He succeeded in causing acids to accumulate in *illuminated* Bryophyllum leaves, by exposing them to gas mixtures containing supernormal concentrations of CO_2, but not high enough to inhibit metabolic processes. We infer that the rate of production of acid was enhanced by raising the CO_2 concentration, and reached a value exceeding the rate of consumption. We have evidence (p. 268) that CO_2 acts as a metabolite in acid production, and believe that, in the light as well as in the dark, the concentration of this gas exerts a quantitative control on acid production. There is no evidence that qualitative changes are brought about by such CO_2 concentrations as occur under natural conditions or as we have used in our experiments.

Again, if relatively high concentrations of CO_2 were maintained in the interior by the unopposed respiration of green shoots in the dark, deacidification, when it sets in as a starvation phenomenon, would be a relatively slow process. Production of acid would go on continuously, as well as consumption. It would depend upon the relative rates of these two processes whether the sap would get more acid or less acid. If, in the light, the accumulated CO_2 is quickly and completely used up by green shoots, photodeacidification would be a quick process. Acid would continue to be consumed, but it would no longer be produced. In support of these statements, we can cite no evidence concerning changes in the composition of the internal atmosphere while acidification in the dark, or deacidification in the light and in the dark, are taking place. We cannot therefore affirm here that the succulent habit indirectly promotes the accumulation of malic acid.

This hypothesis has been formulated in the light of the evidence, recently obtained, that in producing C_4 dicarboxylic acids certain bacteria and animal organs absorb from the outside and assimilate carbon dioxide. The evidence is given on p. 338. It is not impossible that the stages in the production of malic acid from

hexose are (*a*) glycolysis with the formation of pyruvic acid (or of a phosphorylated variety), (*b*) condensation of pyruvic acid with carbon dioxide produced by cell respiration, yielding oxalacetic acid, and (*c*) reduction of oxalacetic acid to malic acid. The partial equations

$$C_6H_{12}O_6 \xrightarrow{-4H} 2C_3H_4O_3 \xrightarrow{+2CO_2} 2C_4H_4O_5 \xrightarrow{+4H} 2C_4H_6O_5$$

satisfy the arithmetical demands of the overall equation written on p. 327. Clearly, there is plenty of scope for experiments on the higher plants to test the validity of this hypothesis.

As was stated on p. 268 Beevers has demonstrated that Bryophyllum leaves exposed in the dark to gas mixtures containing supernormal concentrations of CO_2, absorb this gas while acid is accumulating in them. We claim that this demonstration provides good evidence that CO_2 is a metabolite in the production of acids. Unfortunately CO_2 containing labelled carbon atoms has not been available for carrying out experiments of the kind Wood and Werkman performed on the acid metabolism of bacteria.

(iv.) *Bennet-Clark's hypothesis concerning the control of acid production and consumption.* From his measurements of the changes in the amounts of carbohydrate and malic acid, of the production of CO_2 and of fluctuations in respiratory quotients, Bennet-Clark concluded that malic acid is produced from carbohydrate, and is mainly consumed, not by oxidative catabolism, but by oxidative anabolism with the synthesis of carbohydrate.

$$\text{Carbohydrate} \rightleftarrows \text{malic acid} + \text{energy}.$$
$$\downarrow$$
$$CO_2$$

He proved that in Sedum leaves kept in the dark, whereas at the low temperature of 3° C. the accumulation of malic acid is favoured, at the high temperature of 27° C. the concentration tends to decrease. His results indicated that " the amount of malic acid carbon which is lost from the leaves on warming . . . is clearly not due to its conversion into carbon dioxide, or at most one-third of it is converted into carbon dioxide." He thought that the carbon dioxide came largely from the carbohydrate reserves of the leaves (but see p. 47).

The production of dicarboxylic acids from amino acids, but in relatively small amounts. (i.) *The interconversion of dicarboxylic and amino acids.* Even before Czapek's time it was realized that a fraction of each dicarboxylic acid which is produced or consumed in a plant may come from or be converted into its corresponding amino acid. We have already (p. 48) considered the interconversion of glutamic and α-ketoglutaric acid, and of aspartic and oxalacetic acid.

(ii.) *Acid production exceeds acid loss.* For a period (see Onslow, (*214*)) much weight was attached to Ruhland and Wetzel's hypothesis

that a considerable proportion of the malic, succinic, oxalic and other nitrogen-free acids, which accumulate in some acid plants (*e.g.*, Begonia, rhubarb, but not in the Crassulaceae, see p. 323), arise in the oxidative deamination of amino acids, and that ammonia is simultaneously set free. It was stated that, in the experiments at Leipzig, the production in certain organs of ammonium salts in *high concentration* had been proved. Changes in *total amounts* for the whole plant were not measured.

For example, Ruhland and Wetzel maintained that in their experiments amino acids, passing out from the sprouting rhizome of rhubarb, were deaminated in the growing petioles and there gave rise to vegetable acid (particularly *l*-malic acid) and ammonia. They suggested that the ammonium malate so produced was translocated and used in protein synthesis. Bennet-Clark and Woodruff (*27*), however, measured changes in the *total amounts* per 100 gm. original fresh weight of experimental material of various substances, including total acidity (estimated as malate, although they realized that citrate and oxalate were also present) and ammonium nitrogen, whilst rhubarb rhizomes were sprouting for fifteen days at 23° C. Carbohydrate and nitrogen metabolism associated with growth and respiration were vigorous, but the total acidity remained unchanged. Clear evidence was obtained that acid was translocated from the rhizome to the developing shoot, but none for the new formation of acid, which Ruhland and Wetzel claimed to have shown. The results of another experiment are recorded in fig. 48. Such translocation without new formation would account for the form of the curves A, B, and C during the first fifty days or more of this experiment.

Measuring malate, citrate, and oxalate, Allsopp (*3*), working in Bennet-Clark's laboratory, confirmed these findings. He reported that in the months of March and April in the first season, and before March in the second, the amounts of each of these acids decreased in the rhizome while they increased in the sprouting shoots. Since the total amount per plant of each acid remained the same, he inferred that each was translocated, but that there was no new synthesis during sprouting. The inserted small figures in fig. 49 serve as a summary of Allsopp's results. The fact that unknown acids were present in considerable amounts indicates that knowledge is still incomplete. Inasmuch as fresh weights and, therefore, the volumes of young shoots increase very slowly until late May, acid concentrations may increase greatly in them. Bennet-Clark thinks that Ruhland and Wetzel, by misinterpreting the meaning of this increase, came to an invalid conclusion. For similar reasons relatively high concentrations of ammonium salts may develop in the young shoots; but, from their measurements (see, for example, fig. 48), made over the whole growing period, Bennet-Clark and Woodruff concluded that

"The molar ratios of ammonia/malic acid are small at all times (usually about 0·1), and give no support to the hypothesis that malic acid is derived from the carbon residues of deaminated amino acid. This hypothesis demands that this ratio should be unity or more than unity." With this conclusion Pucher *et al.* (*239*) agree. They measured total acidity and its known components, malate, citrate, and oxalate, as well as ammonium nitrogen, in different parts of rhubarb plants at different stages in development, and reported that ammonium nitrogen was always present in low concentration, but never in concentrations which could be correlated with those of any of the known acids.

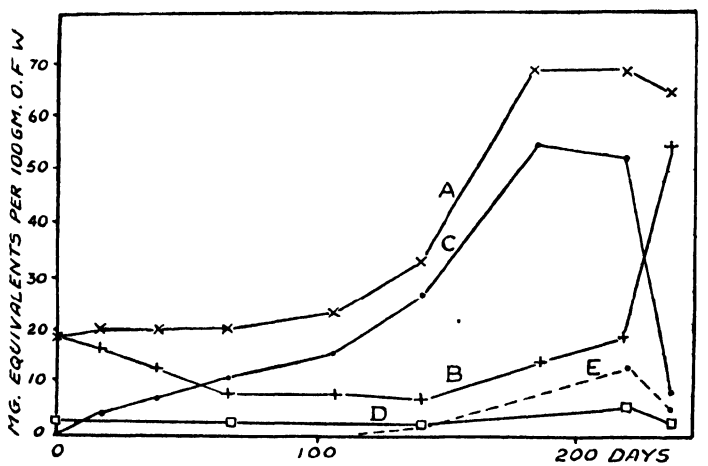


FIG. 48. Seasonal march in total malate expressed as mg. equivalents per 100 gm. original fresh weight for the whole rhubarb plant (curve A), for rhizome plus roots (curve B), and for the shoot (curve C); and the seasonal march for the whole plant in ammonium (curve D) and oxalate (curve E). (From Bennet-Clark and Woodruff, *27*.)

(iii.) *The new formation of acids when photosynthesis is active.* Evidence additional to that already recorded on p. 322 that plant acids are mainly derived from carbohydrate has come from measurements of seasonal variation in the amounts of acid present in whole plants. Bennet-Clark and Woodruff (see fig. 48) did not find any rapid increase in the accumulation of acid in rhubarb until the leaves expanded in the summer, when, of course, photosynthesis would be very active. They proved that *l*-malic acid is then produced. The accumulation of oxalate (curve E) towards the end of the season should be noted.

Allsopp (see fig. 49) has developed this research, and has shown that, in addition to *l*-malate, there accumulate, first in the shoot,

and later in the rhizome, citrate, oxalate and unknown acids. He expressed the view that all these substances are derived from carbohydrate manufactured in photosynthesis.

(iv.) *The production of salts of vegetable acids.* Less than one-half of the total acidity that Allsopp measured was titratable acid, probably because the gradual absorption of mineral salts kept pace with the production of acids. The anions, nitrate, phosphate, and sulphate, would be used in general anabolism, and the residual cations, such as sodium, potassium, and calcium, would be free to combine with acidic metabolic products. Such gradual neutralization of acids should be contrasted with the speedy neutralization that might occur were ammonia to be liberated by some intense local deamination or deamidation. It is known that the production and consumption of ammonium salts (*e.g.*, ammonium malate in rhubarb and ammonium oxalate in Begonia) does occur on a small scale, but Ruhland's hypothesis that they are major controlling events in acid metabolism is, clearly, not acceptable to other workers in this field of study.

Seasonal variations in the production, consumption, accumulation and translocation of acids. The charts in fig. 49 provide convincing evidence of the production and accumulation of citric, malic, oxalic and unknown acids while photosynthesis becomes increasingly active during growth, and after growth has ceased. In drawing conclusions, Allsopp implies that the rate of production of these acids is determined by the amount of available carbohydrate in the cells which produce them.

This view would admit the possibility that in the month of February, when metabolism preparatory to sprouting is in progress, acids might be produced from the carbohydrate reserves in the rhizome. This would account for the increases recorded for some of these acids during this month. Allsopp's results were analysed statistically by Warne, and it was decided that these increases were probably significant. It will be observed that while they were occurring the fresh weight of the rhizome was also increasing, but it has not been determined whether there is any causal connection between these increases. Ideas concerning the consumption of acids are still fluid, but there is evidence that the amounts of the various acids present in the rhubarb rhizome decreased while the fresh weight decreased between October and the date on which the prevernal absorption of water began. Allsopp cites these decreases in acidity as showing that " the acids are playing an active rôle in metabolism and are not merely passive end products as is sometimes supposed."

In the analysis of a tissue what is measured at any time is, of course, the amount of a substance that has accumulated in it. Acids re not necessarily produced where they are found : they may have

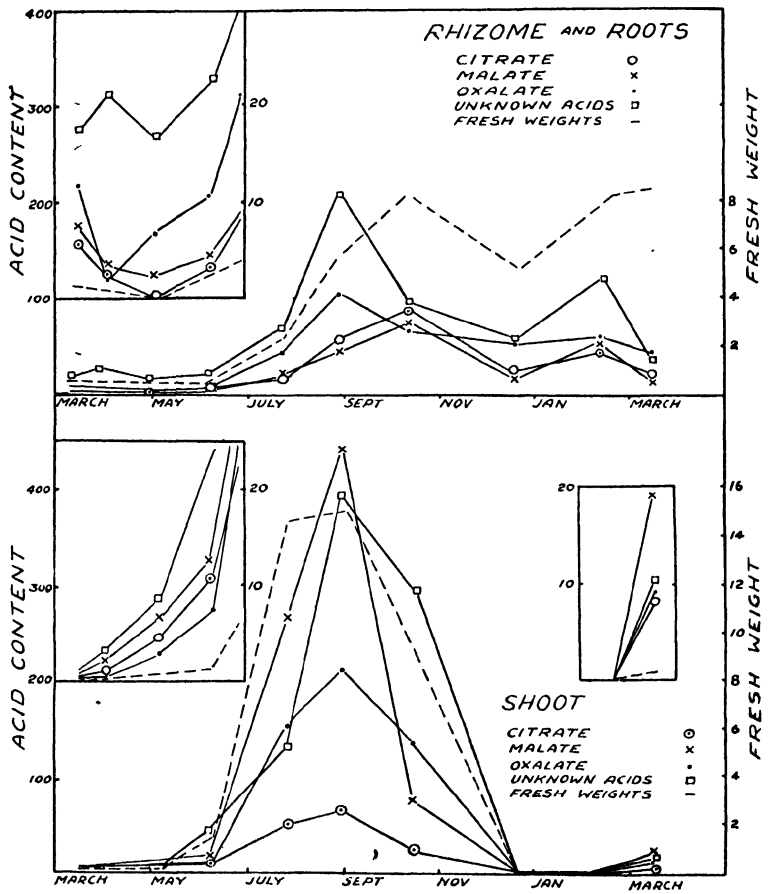


FIG. 49. Seasonal changes in the contents of total citrate, malate, oxalate, and unknown acids, in rhizome plus roots (upper charts) and shoot (lower charts), expressed in mg.-equivalents per 100 gm. original fresh weight (left ordinates). The amount of titratable acid is usually less than one-half the total acidity (p. 315). The inserted figures are portions of the charts on a magnified scale of ordinates, which serve for fresh weights as well as for acid contents: the abscissæ are the same as in the whole charts. The right ordinates of the whole charts refer to the graphs of the changes in fresh weight, and are multiples of the original fresh weight, which is taken as unity. All the experimental data were obtained and recorded by Allsopp (*3*). The plant was rhubarb.

been translocated thereto. The only certain evidence of synthesis *in situ* is provided by the identification of an insoluble salt (*e.g.*, calcium oxalate). The simplest interpretation of the events recorded in fig. 49 as having occurred between May and October is that, for

the most part, all the acids were produced in the green shoots, and there played an active rôle in metabolism. Possibly there was some interconversion among the acids. Allsopp considers that, while acids were being produced and locally consumed, they were continuously migrating from the leaves to the rhizomes, where they accumulated until the early autumn in spite of local metabolic consumption. One acid may also have been changed into another.

An alternative interpretation is that the acids were formed in the rhizomes from carbohydrates translocated from the leaves. But accepting Allsopp's hypothesis, we see that the total amount of acid produced by the green shoots is the sum of the amounts that have accumulated in the shoots and rhizomes, which Allsopp measured, and the amounts that have been consumed, which no one has been able to measure.

F. The Metabolism of Di- and Tricarboxylic Acids by Fungi, Bacteria and Animal Cells

It had been discovered before the end of the last century that acids are among the products of the metabolism of mould fungi and bacteria. For example, it was known that the mould called Citromyces can build citric acid when it is grown in culture solutions containing certain sugars (*e.g.*, glucose), sugar alcohols (*e.g.*, mannitol), or glycerol. Czapek (*67*) expressed the view that these organic compounds were not directly converted into citric acid, but were used in the general constructive metabolism of the mould, and that the tribasic acid was probably derived from protein. He found it difficult to understand how a sugar with a straight chain could give rise to an acid with a branched chain. A modern hypothesis indicates how this may happen (p. 339). He also reported that certain other moulds and yeasts can produce oxalic and succinic acids, and that malic, tartaric and fumaric acids had been detected among the products of fungal metabolism. It was well established that some bacteria produce monobasic acids such as formic, acetic, propionic, butyric and lactic acids, and it has recently been proved that di- and tribasic acids can accumulate in bacterial metabolism and in that of certain animal organs.

We have seen that direction was given to the study of glycolytic carbohydrate catabolism in the higher plants by the results of researches on yeasts and animal tissues. It may well be that knowledge derived from researches on bacteria, moulds and animal tissues, will help to elucidate the biochemistry of the Crassulacean and other types of acid metabolism occurring in the higher plants.

The consideration of and experiments on the acid metabolism of moulds in Bennet-Clark's school. (i.) *Diversity of acid metabolism.* The statement made in the final sentence of the last paragraph is a slight extension of one

made by Bennet-Clark (*21*) when he wrote, " It is assumed by most workers that the chemical mechanisms and biological significance of acid production are essentially the same in fungi as in the higher plants." He marshalled indisputable evidence to show that mould fungi can produce and consume di- and tricarboxylic acids. For example, citric acid accumulates abundantly when a certain strain of *Aspergillus niger* is growing on a medium containing sugar. The acid passes out of the mycelium. At a later stage, when the sugar supply gets short, the acid is reabsorbed and is consumed inside the mycelium. He pointed out the parallel to the increases and decreases in acidity occurring in succulents placed in the dark, and in growing and ripening fleshy fruits. Allsopp (*2*) reported evidence of the production and consumption of oxalic acid in another strain of the same mould. Such evidence may throw light on the acid metabolism of Begonia and other higher plants that produce oxalic acid abundantly.

Bennet-Clark made the broad generalization that, when grown in media containing glucose as the sole source of carbon, Penicillia cause gluconic and citric acids to accumulate predominantly, Aspergilli gluconic, citric and oxalic acids, and Mucors succinic, fumaric and oxalic acids. There are many factors, however, which affect the relative amounts of the different acids found at the time an analysis is made. Among the external factors are the *pH* of the medium, the presence of calcium or other cations which may fix acids, the nitrogen supply and the temperature. Moreover, concentrations change during the course of an experiment. For example, when Aspergilli grow in sugar solutions the acids may reach their maximum concentrations in the order gluconic, citric, oxalic ; but the evidence to be discussed below does not support the possible interpretation that glucose is converted into oxalic acid through the intermediate stages of the production of gluconic and citric acids.

(ii.) *Intermediate metabolism.* The evidence is convincing that mould fungi can readily produce gluconic acid, and di- and tricarboxylic acids from glucose. The production of gluconic acid may be attributed to an oxidation under the agency of glucose oxidase. Müller has shown that *Aspergillus niger* contains this enzyme. The problem of the intermediate metabolism by which a C_6 sugar is converted into a C_6 tricarboxylic acid with a branched chain or into C_4 or C_2 dicarboxylic acids has not yet been solved beyond doubt. Since the acids may be interconvertible it is clearly bound up with that of the metabolic consumption of the acids.

Evidence bearing on these problems has been sought by finding out what substances will serve as sole sources of carbon in the growth of moulds, and by determining which acids are produced during the resulting growth. As Allsopp has pointed out these acids may arise gradually from the glycogen stored during growth and not directly from the substance providing the sole source of carbon. Bennet-Clark, however, was satisfied that feeding experiments had proved that moulds can build up by not too circuitous a route C_4 acids and citric acid from certain C_2 compounds (*e.g.*, acetic acid), which might well arise by glycolytic cleavage during growth under natural conditions. It is recalled that Ehrlich attributed the accumulation of succinic acid in alcoholic fermentation by yeast to the deamination of aspartic acid. Bennet-Clark admits that a fraction of the di- and tricarboxylic acids may be produced thus, but strongly supports the view that in the fungi, as in the higher plants, these acids arise for the most part in carbohydrate rather than in protein metabolism. Indeed, he interpreted his experiments by supposing that not only are the acids produced from carbohydrates, but that they may themselves be converted into carbohydrates by a process " somewhat similar to the oxidative anabolism

of Blackman or the Meyerhof cycle." The classical view concerning the consumption of these acids is that they undergo interconversion or are aerobically oxidized with the ultimate production of carbon dioxide and water.

(iii.) *Feeding experiments and the consumption of acids.* Bennet-Clark and La Touche (*26*) grew *Aspergillus niger* in a nutrient solution containing glucose, and measured the respiration. Over a week later they replaced the nutrient solution by pure water. During the following period the glycogen stores in the fungus decreased, and the plotted *respiration time* curve showed a logarithmic fall to a fairly steady value. In one experiment glucose was then fed to the starving mycelium. The respiration rose to its original value, and oxalic acid accumulated in the medium. In another experiment the starving fungus was fed with two per cent. citric acid. Consumption of the acid was rapid at first, and the amount of titratable acid declined ; none was even partially converted into oxalic acid. Inasmuch as respiration was not stimulated, the persisting CO_2 output was attributed to the oxidation of carbohydrate at the expense of the stored glycogen. The inference made was that citric acid can be consumed without being oxidized to CO_2 or converted into another acid. Further experiments indicated that this inference could be extended to cover the consumption of malic, glycollic and oxalic acids. The accumulation of ammonium salts, amides, and esters, was negligible in comparison with the amounts of acid consumed. The only remaining explanation of consumption appeared to be a reduction to hydroxyaldehydes with the final production of carbohydrate. Sampling errors were too great for evidence to be obtained by the crucial quantitative experiment of determining whether glycogen increased at the expense of absorbed acid. The fact was established, however, that glycogen may accumulate when a mould grows in a medium containing citric acid as the sole source of carbon.

When citric acid was disappearing the measured respiratory quotient reached a maximum of about 1·75. The real R.Q. for the oxidation of carbohydrate is unity, and 1·33 for the complete oxidation of citric acid (of which no evidence was found). The empirical equation

$$C_6H_8O_7 + H_2O = C_6H_{10}O_5 + {}_3O$$

was suggested to explain how atmospheric oxygen might be spared by the conversion of citric acid into glycogen. Accordingly the high value of the quotient was cited as evidence of this conversion. Sugar undergoes respiratory oxidation. All the CO_2 produced is given out and measured, but only a fraction of the oxygen consumed comes from the outside air, because there is an intracellular supply. The greater this supply the higher will be the measured respiratory quotient. In one experiment the value varied : 1·75 was the maximum.

Clearly Bennet-Clark has made a good case for his hypothesis of the conversion by starving mycelia of acids into carbohydrate, but it is possible that well-nourished cells living under natural conditions may display additional powers. In his articles Bennet-Clark often points out that vegetable acids may provide the carbon skeletons of a number of compounds, *e.g.*, certain amino acids and alkaloids.

(iv.) *The metabolism of oxalic acid.* There are many unsolved problems relating to the production of oxalic acid. One array of facts to be explained is that *Aspergillus niger* has been grown with the production of oxalic acid when fed with sodium salts of succinic, fumaric, malic or acetic acids, but without the accumulation of oxalic acid when the sole source of external carbon was in the sodium salts of lactic, pyruvic, tartaric, malonic, glycollic

or glyoxylic acids. Allsopp (*2*) thinks it is possible that oxalic acid is often derived from glycogen reserves and not directly from the acid fed to a mould. He showed that the accumulation of oxalic acid by *Aspergillus niger* growing in glucose solution, was greatly retarded by adding lactic acid, and depressed by adding pyruvic acid. He concluded that when oxalic acid is not produced from glycogen it is because the acids fed to the mould differentially inhibit this particular conversion. His general conclusion was that, when no such inhibition is operating, oxalic acid is in reversible equilibrium with carbohydrates present in the mycelium. We have already noted that Bennet-Clark and La Touche, having failed to find any other satisfactory explanation of the disappearance of oxalic acid fed to starving *Aspergillus niger*, attributed it to the conversion of the acid into carbohydrate by oxidative anabolism.

In their experiments oxalic acid did not appear when starved mould was fed with citric or glycollic acids, and Allsopp, using a similar technique, found that the consumption by starved mould of oxalacetic, malic, succinic and pyruvic acids, could not be attributed to the production of oxalic acid. Inasmuch as this fungus can produce oxalic acid readily from certain hexoses and pentoses, and also from gluconic acid, Allsopp was justified in doubting whether this production was either by way of glycolysis (*i.e.*, with the intermediary formation of pyruvic acid) or via citric and C_4 acids. His evidence that oxalacetic acid disappeared rapidly without the accumulation of oxalic acid, and that sodium oxalacetate was decomposed almost entirely into sodium pyruvate and sodium carbonate is antagonistic to the attractive hypothesis that oxalic acid is produced by the hydrolysis of oxalacetic acid. Allsopp considered that oxalic acid may arise by the oxidation of the two end carbon atoms of a molecule with at least five atoms in the carbon chain. He regards gluconic acid as an intermediate metabolite when glucose is being converted into oxalic acid. Presumably the possibility cannot be excluded that in moulds, which are believed to be so adept in producing glycogen from C_2, C_4 and C_6 acids, pentoses are converted into hexoses before oxalic acid is formed.

New evidence concerning the biochemistry of acid production. Among the hypotheses considered by Bennet-Clark *et al.* and by Allsopp for the production of di- and tricarboxylic acids from carbohydrate were (i.) that hexose undergoes glycolysis, yielding C_2 compounds which condense to build C_4 and C_6 acids, and (ii.) that hexose is oxidized to produce gluconic acid, which, by non-glycolytic cleavage, yields a C_4 compound and oxalic acid. These hypotheses served to explain many of the facts known when Bennet-Clark wrote his review on acid metabolism. They have not been discredited, but since 1935 other hypotheses have been put forward.

Wood and Werkman (see p. 45) discovered that propionic acid bacteria, in producing propionic and succinic acids with glycerol, mannitol or certain other substrates as the sole organic source of carbon, assimilate carbon dioxide from the air. They found that "there was a net decrease in CO_2 of the system and in amounts equivalent to the succinic acid formed," and that little or no succinic acid is produced in the absence of carbon dioxide. They established beyond reasonable doubt the fixation of carbon dioxide by experi-

ments in which they supplied the bacteria with gas much richer in C^{11} or C^{13} isotopes than is ordinary carbon dioxide. The decay of the C^{11} isotope, which is radioactive, proved too rapid for satisfactory work, but convincing results were obtained with gas containing the stable isotope C^{13}, heavy carbon. In many of the succinic acid molecules synthesized, one of the carboxylic groupings contained heavy carbon. They concluded that a C_4 dicarboxylic acid can be produced by the union of a C_3 compound and carbon dioxide.

When carbohydrate serves as the source of carbon, the C_3 compound which condenses with carbon dioxide may arise during glycolysis. Were the hypotheses formulated diagrammatically in fig. 1 to be substantiated, the synthesis of any one C_4 acid from a product of glycolysis would lead to the production of the others, members of the citric acid group, certain amino acids, and, probably, other substances. It is possible, therefore, that the reaction we are considering may turn out to have extreme importance.

In their review Werkman and Wood (*329*) distinguish with excellent clarity between plausible inference and what is established by experiment. Some years ago they suggested, without, as they admit, any experimental proof, that the fixation reaction might be the production of oxalacetic acid by the condensation of pyruvic acid and carbon dioxide. A phosphorylated variety of pyruvic acid may be the actual reactant. In the equation written below it is shown where the labelled carbon atom is believed to go, when carbon dioxide containing heavy carbon is used for the reaction. If the number 13 is left out, the same equation will represent what is postulated to occur in ordinary air.

$$CH_3CO.COOH + C^{13}O_2 \rightleftarrows C^{13}OOH.CH_2.CO.COOH$$

The reversible sign is used because it is known that, under certain conditions, oxalacetic acid can be decarboxylated with the production of pyruvic acid (see, *e.g.*, Allsopp's experiment, p. 337). In 1941 Krampitz and Werkman effected this change with an enzyme prepared from *Micrococcus lysodeikticus*. Enzymic synthesis *in vitro* of oxalacetic acid from pyruvic acid and carbon dioxide was not achieved: had it been, proof as decisive as any a biochemist can obtain would have been secured that oxalacetic acid is involved in the fixation reaction. Strong presumptive evidence of this was, however, obtained when these two workers enzymically decarboxylated oxalacetic acid containing ordinary carbon in the presence of $C^{13}O_2$. They found that during the course of the reaction molecules of oxalacetic acid containing heavy carbon had a detectable existence. They inferred that some synthesis was occurring in opposition to decarboxylation throughout the experiment. In the final state no oxalacetic acid remained.

The phenomenon of carbon dioxide fixation is not confined to

propionic bacteria. By carrying out quantitative experiments on the absorption of carbon dioxide and the production of acids, and by performing experiments with labelled carbon atoms (usually heavy carbon), several investigators have been able to trace the carbon coming from the absorbed gas in the succinic acid produced by *Escherichia coli*, *Aerobacter*, *Streptococcus*, and other micro-organisms. Moreover, in some of these organisms the labelled carbon has also been found in malic, fumaric and aspartic acids. Hypotheses covering the formation of all these acids from oxalacetic acid are included in fig. 1.

Evidence has been obtained (see Krebs, *177*) that Saccharomyces, Aspergillus and Mucor may require carbon dioxide for growth, that *Rhizopus nigricans* produced fumaric acid containing a labelled carbon atom when it was cultured on a glucose solution in the presence of $C^{11}O_2$, and that, under similar cultural conditions *Aspergillus niger* synthesized citric acid containing radioactive carbon. No reports have been seen of the appearance of a labelled carbon atom in oxalic acid. It will be observed that the production of this acid is not accounted for in any of the reactions summarized in fig. 1. So far it has not been reported as a product of the metabolism of animal cells or bacteria.

By similar methods it has been shown that some animal tissues can assimilate carbon dioxide. Among the facts established is that, with the fixation of carbon dioxide, dicarboxylic acids (*e.g.*, α-ketoglutaric, *l*-malic, fumaric and succinic acids) and tricarboxylic acids (*e.g.*, citric acid) may accumulate. Oxalacetic acid has not been detected. It is known that this acid can be very rapidly converted into other C_4 acids and citric acid. The conclusion drawn is that it does not accumulate because its rate of consumption is greater than its rate of production. In the isotope experiments on pigeon liver it was found that a labelled carbon atom was present in one of the carboxylic groups of each of the acids synthesized.

The biochemical mechanism of the synthesis of citric acid from the products of glycolysis and the assimilated carbon dioxide is still under consideration. We recall (see p. 334) the difficulty Czapek found in seeing how a branched chain acid could come from a sugar, and point out that in fig. 1 one possible solution of this difficulty is given. It has been reported that under the agency of an enzyme called citrogenase, which occurs in kidney, oxalacetic acid will condense with pyruvic acid, and certain other ketoacids, to give a tricarboxylic acid. The hypothesis contained in fig. 1 may not be far from the truth. Another idea that has been favoured is that citric acid may be formed by the condensation of oxalacetic acid and acetic acid or acetyl phosphate, which contains an active phosphate bond (p. 58), and so may provide energy for the synthesis.

Evidently the stage is set for new investigations on the acid metabolism of the higher plants. In attributing the accumulation of acids in succulent plants placed in the dark to a reaction between respiratory carbon dioxide and products of the glycolysis of sugars photosynthesized on the previous day, the hypothesis put forward on p. 327 may possibly be allowed to have the merit of suggesting that a very old problem concerning the acid metabolism of higher plants should be considered in the light of new knowledge about a similar although not necessarily an identical problem, which has been gained by researches on other organisms.

PART IV

GROWTH AND MOVEMENT

CHAPTER XVI

GROWTH

A. Primary Growing Regions [1]

GROWTH may be defined as a permanent change in volume, which is usually accompanied by a change in form. This definition is applicable to diminution in volume, such as occurs in the contractile roots of the common arum and crocus, as well as to the increase in volume with which growth is generally associated. We shall, however, confine our attention to members that increase in length as they grow, and summarize such facts as have been won by microscopical observation and experiment, with a view to indicating that growth and development are the result of the complex interplay of many metabolic and biophysical processes in the regions where cells multiply, enlarge, and differentiate.

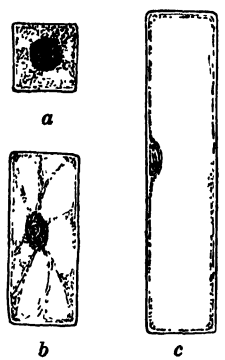


FIG. 50. (See text.)

Primary meristematic regions. These formative tissues, composed of non-vacuolated living cells (fig. 50, *a*), are located at root tips and shoot apices. They are centres of intense metabolic activity (*cf.* p. 180) where food materials are (*a*) assimilated to form new protoplasm (the specific syntheses, governed by the heredity of the organism, of proteins, lipoids, nucleic acid, etc.), and (*b*) oxidized in respiratory processes which provide energy for the anabolic events indicated in (*a*). The increase in the amount of protoplasm is accompanied by mitotic nuclear division, and cell division is completed by the formation of new cell walls, which are produced as a result of carbohydrate anabolism. The new cells swell owing to the imbibition of water by the protoplasmic gels.

Regions of primary enlargement. These regions are readily recognized by making marks with Indian ink on the growing member, and observing where the marks separate during growth (*e.g.*, fig. 51). In a root the region of enlargement is localized near the tip, but in a

[1] Secondary growth following the activity of cambium or phellogen might be treated in a similar fashion.

stem it is more extensive and may be distributed over several internodes below the apex. In certain monocotyledonous leaves (*e.g.*, onion leaves) growth is confined to basal portions, but in most leaves it is more distributed. In these regions new cells, which have been formed in the meristems, absorb water by imbibition and osmosis, and, owing to the plastic extensibility of the cell walls, undergo turgor expansion and become vacuolated (fig. 50, *b* and *c*). The absorption of the water necessary for turgor expansion may be determined by an increase of osmotic pressure resulting from hydrolyses, *e.g.*, of starch to sugar, or by an increase in the extensibility of cell walls such as may be brought about by the action of auxins on growing cells. Expansion is accompanied by the intercalation in and deposition on pre-existing lamellæ of new cell wall substances. Thus carbohydrate metabolism plays a noteworthy part in primary enlargement. Respiratory oxidations provide energy and cellulose and other substances are synthesized.

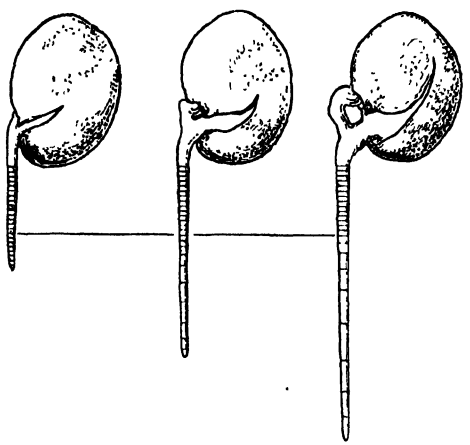


FIG. 51. The regions of primary enlargement in a root.

The degree to which a cell can expand when situated in a growing tissue is restricted by the magnitudes of the wall pressure of the thickening cell wall and of the opposing hydrostatic pressures of neighbouring cells. Strips cut from newly grown tissues curve outwards (see p. 471), showing that during expansion, dermal tissues tend to become stretched, and tissues towards the interior compressed. These tissue tensions help to determine the external form of the growing member: as cells mature this form becomes permanently established.

Maturing primary regions. In these regions cells differentiate to form the permanent primary tissue elements. Maturation begins during enlargement and usually continues after turgor expansion has ceased. Very varied forms of metabolism govern the specific processes of maturation (p. 181). The racial characters inherited by the individual, and the environmental conditions together determine the fate of the food substances that are assimilated, and hence the forms that will be assumed by the developing individual. Aerobic respiration is, of course, vigorous.

B. Metamorphosis

An adult organism, grown under any environmental conditions, will exhibit certain characters and modes of behaviour, whose appearance and development may be attributed to the action of the internal factors that constitute the inheritance the individual derives from its parents. Nevertheless, identical twins grown under different environmental conditions may possess strikingly different characteristics. Thus environmental factors may affect the size, form and orientation of plant members, or alter the rates at which the various phases in the life cycle are passed through. For example, plants grown under arid conditions, in soils deficient in one or more of the essential elements in available form, at low temperatures, or under other conditions unfavourable for growth, will, when fully grown, be stunted by comparison with plants of the same race which have grown under favourable conditions. The environment governs in part, therefore, the size attained by a plant. There is, however, a limit of size set by its inheritance beyond which the plant cannot grow even under optimum conditions.

The term *stimulus* has already been defined (p. 4). The mode of action of some of the environmental factors that govern growth, development, and configuration, appears to conform to the requirements of that definition. Such factors may be described as formative and orientative stimuli. The latter group will be considered in chap. XVI, and a few examples of the modifying effects of formative environmental stimuli will be given here.

The formation of chlorophyll is a metabolic event brought about by protoplasmic systems that are under the control of hereditary factors, but leaves of seedlings that have inherited these factors do not turn green if iron salts are not available in the environment. After comparing the yellow-leaved plants, which are called chlorotic plants, with normal plants grown in the presence of iron salts, one infers that iron salts constitute a formative chemical stimulus. In the presence of iron salts but in the absence of light from the blue-violet end of the spectrum, chlorophyll formation does not as a rule occur. Hence we infer that light acts as a formative stimulus in the growth of green plants. Plants grown in the dark are described as etiolated plants, and, besides lacking chlorophylls *a* and *b*, exhibit other striking differences from normal plants. For example, in etiolated bean plants, grown in absolute darkness, the plumule remains hooked, the lateral leaves do not develop, the internodes are relatively long, and several peculiarities of anatomical structure, such as the reduced formation of lignified tissue, and the presence of a Casparian band in the endodermis, have been reported (Priestley, *236*). It is thus clear that many of the characters of a normal plant result from the interplay between internal factors and the stimulus of light. Highly complex problems confront those who attempt to analyse the sequence of changes that are initiated by the perception of the stimulus of light, and lead to the development of so many different characters.

Heat energy often acts as an external stimulus. Thus the colour of the flowers of *Primula sinensis* var. *rubra* depends upon the temperature at which the flowers develop. At ordinary temperatures the flowers are red, and we infer that they carry an inherited mechanism that can bring about the formation of anthocyanin. If, however, the plants are grown in a hothouse, the flowers produced are white. Clearly the behaviour of the inherited mechanism is governed by the temperature of the environment.

Among other external stimuli that may have a modifying influence during growth is the external moisture. According to Palladin (*223*)

(p. 269), plants growing in dry regions often possess spines, and if such plants (*e.g.*, *Genista anglica*, described by Livingston as "broom") are grown in a very moist atmosphere the spines are generally replaced by short leafy branches.

C. A Note on the Causal Conditions for the Production and Perpetuation of Adaptations

In analysing growth phenomena we are concerned not with final or purposeful causes, but with proximate causes, and look upon all plant structures as the inevitable end products of the reactions between inherited internal factors and significant environmental factors. The end products may or may not be adaptations, *i.e.*, members possessing external form or internal structure which render the whole plant peculiarly fit to survive in a particular environment. It should be realized that even when the use of the term adaptation is justifiable, it is not justifiable to assign as the cause of the formation or occurrence of any structure its importance to the plant as is so often done in sentences such as "the *reason* for the production of chlorophyll is that plants require light energy for photosynthesis." Chlorophyll formation is a complex event, and we do not yet know the nature of all the inherited internal and the necessary external causal factors concerned. What one may legitimately state is that "as a *result* of chlorophyll production, green plants absorb light energy and carry out photosynthesis."

Nevertheless, such adaptations towards a given environment as are inevitably formed during the development of a given individual will have survival value for the race to which that individual belongs. The persistence of a race (*e.g.*, Genista) in a specified environment (dry banks in the open) will be favoured, because environmental factors and inherited internal factors will in every succeeding generation interact to produce grown plants (Genista bushes with spinous green branches, and other xeromorphic characters) which are adapted to conditions in that environment.

Bearing in mind the intense competition in the field for space, light, etc., and the antiquity of existing plant species, we are not surprised to find that thriving plants show growth forms and internal structures that may be described as adaptations to their environments.

D. The Classification of the Functional Anatomical Systems in Plants

As a result of the interplay of factors of inheritance and environmental factors, tissue systems are produced which exercise functions in the welfare of the whole organism, and hence of the race to which it belongs. A classification of these functional systems is given below.[1]

Metabolizing systems. All living cells are metabolizing systems (*e.g.*, they all respire), and the following different types are frequently distinguishable :—

(*a*) The zygotes produced by the fusion of gametes. These are formative systems which can assimilate foods into their own kind of nucleated protoplasm. Nuclear division and cell division follow, and new cell walls are formed.

(*b*) Meristematic tissues (i.) primary meristems at apices of stem and

[1] The classification has been developed from that proposed by Haberlandt (*109*).

root; (ii.) cambium, phellogen, and sometimes pericycle; (iii.) reproductive tissue which gives rise to microspores and megaspores, and finally to male and female gametes.

(*c*) Enlarging and differentiating tissues in any region. These are formative systems which assimilate and transform foods into the substances composing the structures of the permanent primary and secondary tissues.

(*d*) (i.) Photosynthesizing tissues (any living tissue containing chloroplasts). Primarily these tissues manufacture sugars in the presence of light. They also manufacture from raw materials other food substances independently of the direct influence of light. (ii.) Storage tissues (vacuolated parenchyma of shoot and root, and sometimes of specialized parts such as seeds, tubers, rhizomes, corms, and bulbs). Here, carbohydrates, fatty oils, and proteins, accumulate singly or together, and are drawn on in a later phase of vegetative activity. (iii.) Feeding tissues. Two types may be distinguished, viz., the nectaries of flowers, which function in pollination; and the parenchyma of fleshy fruits, which function in seed dispersal. In both types metabolism leads to the production of the food substances that attract the visiting insects or birds. (iv.) Secretory tissues. Glands secreting resins, ethereal oils, and mucilages, and the nectaries of flowers, may be regarded as specialized metabolic systems when they produce the secreted substances. (v.) Specialized nutritive tissue, *e.g.*, tapetum. (vi.) Pigmented attraction tissues in coloured members of inflorescences whose flowers are pollinated by insects, and in coloured fleshy fruits.

Absorbing and eliminating systems. (*a*) The piliferous layers of roots. These absorb water and dissolved mineral salts and oxygen, and give off carbon dioxide.

(*b*) Parenchyma with wet cellulose walls in contact with intercellular air. These absorb and eliminate matter in the gaseous state. The activities displayed depend upon the nature of the cells and the environmental conditions. (i.) Photosynthesizing tissues in the light absorb carbon dioxide and eliminate oxygen. (ii.) Photosynthesizing tissues in the dark and all other parenchyma in the light or the dark absorb oxygen and eliminate carbon dioxide. (iii.) All parenchyma abutting on intercellular spaces, and thinly cuticularized epidermal cells in contact with the outside air, eliminate water as vapour.

(*c*) (i.) Superficial specialized glands and nectaries secrete water and substances in solution directly to the exterior. (ii.) Turgid unspecialized cells or internal glands secrete water and substances in solution into intercellular spaces; these liquids may then pass through ordinary stomata or specialized water pores to the exterior. (iii.) Mucilage-, oil-, resin-, and gum-passages, raphide sacs, etc., serve as internal excretory reservoirs.

Conducting systems. (*a*) In the conducting parenchyma of pith, cortex, medullary rays, xylem, phloem, and the bundle sheaths of leaves, water and solutes in crystalloid solution move slowly by osmosis and diffusion respectively.

(*b*) In xylem vessels and tracheides, water and inorganic substances move rapidly in all directions. These tissue elements sometimes conduct upwards soluble food materials from storage tissues.

(*c*) In sieve tubes (which are always associated with companion cells) food materials are rapidly translocated from photosynthesizing and storage systems to regions of utilization.

Dermal systems. (*a*) Cuticularized epidermal layers protect living cells in the interior from injury from outside. They are exceedingly important in restricting water loss from turgid primary tissues.

(*b*) Suberized cells of cork tissue are also protective in function, and restrict water loss from secondary turgid tissues.

(*c*) Root caps protect the meristematic regions of roots from injury by the soil particles during growth.

(*d*) Hairs may reduce the rate of water loss, and hairs and emergences may have a protective function.

Ventilating systems. Intercellular air systems with their external openings of (*a*) stomata associated with epidermis (note that cuticle is impermeable to gases); (*b*) lenticels associated with cork tissue (note that cork is impermeable to gases).

Mechanical systems. The following tissue elements contribute to the rigidity of shoot systems :—

(*a*) Turgid cells within the stretched epidermis of young shoots.

(*b*) Collenchyma and sclerenchyma of primary tissues.

(*c*) Lignified elements, particularly sclerenchyma [1] of secondary tissues. The vessels and tracheides in old wood in trees sometimes become filled with substances that harden, and so increase the rigidity of the tree trunk.

Motor systems. (*a*) Living and non-living tissues which govern the dehiscence of anthers and of certain fruits.

(*b*) Specialized parts (*e.g.*, tendrils) or unspecialized parts that are capable of differential growth. In these and only in these can growth curvatures occur.

(*c*) Specialized pulvini, in which alterations of turgor cause movements of variation.

Sensitive systems. In this class are placed tissues (*e.g.*, geoperceptive root tips and photoperceptive coleoptile tips) that are supposed to be peculiarly sensitive to external stimuli.

Stimulus-transmitting systems. Paths for the travel of stimuli must exist in plants when it can be shown that regions of perception and motor systems are separated.

E. The Integration of the Activities of Functional Systems within the whole Organism

Simple summation of functional processes. A couple of examples will make it clear that the functional systems classified in section D form an ordered aggregate when co-existing in the whole organism. There is a mutual relevance among associated physiological processes. First we note that the continued efficiency of green leaves as organs of photosynthesis depends on the proper and simultaneous action of water-absorbing, conducting and ventilating systems; secondly, that the rate of water absorption is governed by the concentration in the root sap of soluble organic matter manufactured in and transported from green leaves, and by the rate of transpiration. Many other examples of this type of interdependence might be given, and it is possible that if we knew the functional powers of each related part

[1] It should be noticed that sclerenchyma, xylem vessels and tracheides, cork tissue and lenticels, and intercellular spaces, are non-living but functionally essential parts of living plants. The vital powers of growth, development, etc., reside in the living cell, but are dependent upon the continued functioning of the above-named non-living parts.

considered as a separate unit, we could predict how the whole plant would behave. Activity of the whole plant would then represent the simple summation of the activities of the parts. The effects of such association of processes within the whole are not qualitative but quantitative.

Correlations. For many years it has been recognized, however, that the properties of a whole organism may differ from the numerical aggregate of the properties possessed by each of the constituent functional systems. Powers not previously displayed may be revealed, or others previously shown may be modified or suppressed, by the association of parts within the whole organism. In some way, therefore, events in one part of an organism may exert a qualitative as well as a quantitative effect on the behaviour of another part.

Goebel (1880) used the term *growth correlations* to describe those forms of mutual influence between distinct parts of a plant which determine the properties of the parts within the whole.[1] The protoplasm in a given cell may possess very varied powers, and the activities actually developed may depend upon the incidence of stimuli belonging to the external environment (section B) or, alternatively, on internal stimuli generated elsewhere within the plant body. Correlative behaviour is, in a sense, autonomic response to internal stimuli.

Growth itself may be regarded as an autonomic response to an internal stimulus. Thus, apart from the processes of cell division, which may be attributed to the persistence of the stimulus of fertilization, cell enlargement is dependent upon the transmission of stimuli to the enlarging cells from other regions (section H). Another excellent example is afforded by the striking changes that often take place in gynœcial or receptacular tissue after fertilization. Also there is evidence that growing buds and leaves provide a cambial stimulus which promotes cell division in the cambium below them (Snow, *275*).

Many examples could be given of correlative readjustments following injury. These often have survival value for the plant in its modified state. If the main root is destroyed, one or more lateral roots, normally plagiotropic towards the stimulus of gravity, may become positively geotropic, and so promote the downward growth of the whole root system. It would appear that the presence of the main root in the whole plant exercises some influence on the geotropism of the lateral roots. Influences of a similar kind appear to affect the geotropism and mode of development of the lateral buds of spruce. In normal trees, the lateral buds give rise to dorsi-ventral shoots which are plagiotropic towards gravity. If, however, the terminal bud is killed, at least one of the lateral buds may develop into a

[1] For a masterly discussion of the pioneer work on this difficult subject, see Jost (*158*).

negatively geotropic shoot possessing radial symmetry. One further example of modified behaviour following injury must suffice. It has been shown that if the aerial shoot of a potato plant is cut off, one of the subterranean buds, which normally would give rise to a tuberiferous shoot, develops into a new aerial shoot.

In contrast with such correlations between tissues as lead during normal ontogeny to new, augmented, or modified activity are those which have inhibitory or retarding effects. Thus there is evidence that mature cells containing nucleated protoplasm may retain the power of cell division. This power is not normally displayed in specialized tissues, and only becomes apparent after some form of injury or other stimulus has altered the internal relations within the whole. For example, our ability to propagate plants by cuttings, or by grafting, and the power of plants to heal wounds by forming cork or callus, depend on the fact that, under the altered conditions, specialized tissues develop meristematic activity. Why then does cell division not normally occur in these tissues ? The answer given to this difficult question by supporters of Goebel's views is that, in the normal whole plant, cell division is correlatively inhibited, *i.e.*, it is held in abeyance by the influence of related parts. It is also supposed that correlative inhibition is responsible for the fact that certain winter buds may remain dormant for a number of years. When for some reason the inhibitory influence is removed, these buds develop into leafy shoots. The behaviour of the buds on the epicotyl of seedlings of *Phaseolus multiflorus* or *Vicia faba* provides a less complex system for experimental investigation, and it appears that events in the apical tissues lead to the inhibition of the growth of lateral buds, for these buds may develop into shoots if the apex is cut off (Snow, *276* and *277*).

Protoplasmic connections, and chemical stimuli in the integration of plant behaviour.[1] " To refer so numerous and heterogeneous phenomena to the principle of correlation is only a step towards explaining the causes of plant form, and that only a slight one " (Jost, *158*, supplement, p. 100). For deeper insight one must face questions such as : what tissues are in correlative association ? which, among these tissues, generate the internal stimuli, and which respond to such stimuli ? what is the nature of these internal stimuli, and along what paths do they travel ?

In considering the last question, great importance was for many years attached to the observed fact that the protoplasm of contiguous living cells may be connected by threads which traverse the cell walls. Pfeffer (*228*) expressed the view that " from general physiological considerations the attainment and maintenance of harmonious co-operation throughout the plant by the intercommunication of

[1] See also pages 404 and 440.

stimuli renders the existence of living continuity so absolutely necessary that had it not already been discovered its presence must have been assumed, for in no other way could the observed phenomena have been explained." Moreover, he suggested that protoplasmic threads may act as the channels along which stimulating influences flow, and considered the possibility that these influences were chemical. Even earlier Sachs (1882) had suggested that "root-formation in cuttings of stem and root is due to the downward travel of root-forming substances," and this chemical view persisted. Jost (1907) believed that stimuli might frequently be chemical in their nature, but admitted that such internal chemical stimuli were then quite unknown.

In more recent years experimental evidence (see next chapter) has been secured for the existence in plants of chemical substances which regulate growth in length, cambial cell division, root formation, and the inhibition of bud development. It may be that many of the phenomena reported in this section will be explained in terms of the promoting, modifying, or inhibiting properties, of internal chemical stimuli or hormones (see p. 374). Haberlandt (1913) found that cell division leading to cork formation, which occurs after certain plant organs (*e.g.*, potato tubers) have been injured, was dependent upon the generation of a wound hormone. He showed that this substance, unlike an enzyme, was thermostable. Snow (*276* and *277*) found that the influence inhibitory to the growth of the lateral buds of *Phaseolus vulgaris* (see p. 348) can travel from the apical tissues of the epicotyl to the lateral buds across a moist protoplasmic gap or along a zone of stem killed by scorching. Snow (*275*) also showed that the cambial stimulus "can pass across a protoplasmic discontinuity, and even through an interposed piece of moist linen."

The trend of this earlier work suggested that continuity of protoplasm throughout the plant is not always an indispensable condition for the integration of separate activities, and more recent work supports this conclusion. Nevertheless it still remains possible that protoplasmic connections play a part in the phenomena which are classed as correlations. It should be clearly realized, however, that no plants possess a differentiated nervous system, and that no experimental evidence exists which would justify the description of correlations in plants in terms used in describing nervous correlations in the higher animals.

F. The Necessary Conditions for Growth

Experimental. The necessary conditions for growth are readily demonstrated by experiments on the germination of seeds. It is usually found that after a few days the percentage germination of pea seeds supplied with water and air, and kept at 20° C., is high, but no

seeds germinate in the absence of water or oxygen, or if kept in an ice box or in an incubator at 50° C. in the presence of water and air. The seeds as a rule fail to germinate (*a*) in decinormal acid or decinormal alkali (*i.e.*, the *pH* of the medium must be suitable), (*b*) in solutions of high osmotic pressure, or (*c*) in atmospheres containing large amounts of carbon dioxide. Light is sometimes an important factor, but pea seeds germinate either in the light or in the dark.

In order to judge whether failure to germinate is due to injury or merely to the arrest of growth processes, ungerminated seeds should at the end of the experiment be transferred from the inhibiting conditions to conditions known to be favourable and the subsequent percentage germination determined. For instance, seeds that have failed to germinate in nitrogen should be exposed to air at ordinary temperatures, and those from the ice box or the hot incubator to a temperature of 20° C. Where effects are injurious the time factor (p. 283) will have great significance. Exposure for several days to nitrogen may be endured, but longer exposure may prove lethal; or the seeds may withstand temperatures of 40° C. for a few hours, but be killed after longer periods.

From the results of experiments on seed germination or on plant growth (*e.g.*, water-culture experiments, such as are described on p. 166), carried out under clearly defined conditions, and from observations such as those recorded in section A, we may infer that certain internal and external conditions must be satisfied before growth can occur.

Necessary internal conditions. (*a*) There must be present *meristematic tissue* in an *active* state. It is well known that certain seeds and winter buds appear to pass through a dormant period. This may not be true dormancy. Thus the germination of seeds may be arrested because (i) external conditions are not suitable for germination (*e.g.*, the temperature, or the water content of the soil, may be too low); (ii.) the action of certain of the essential factors is impeded by the seed coat (*e.g.*, the coat may be impermeable to water or more rarely to oxygen); or (iii.) the seed coat exerts mechanical resistance to the emergence of the radicle. In true dormancy certain necessary changes must occur in the interior before growth will continue. Experimental investigations in the United States indicate that many seeds (particularly of the Rosaceae) must pass through an after-ripening period before they will germinate. Sometimes exposure to low temperatures, such as are prevalent during cold periods in winter, is a necessary experience. Germination itself, however, is favoured by warmth. For summaries of work on dormancy and the germination of seeds, see Macgregor Skene (*269*), West (*330*), Crocker (*63*), and on the dormancy and methods used in the breaking of dormancy of winter buds, see Stiles (*291*).

(*b*) The plant must contain available *food reserves* (proteins, carbohydrates, fats, mineral salts, water) on which to draw, as it does in germinating seeds, sprouting buds, etc., or possess differentiated functional tissue elements which absorb raw materials and elaborate them into foods, as must happen in independent seedlings with green leaves.

(*c*) A state of turgor must exist in living cells, *i.e.*, the supply of water to the growing tissue itself must be such that the tissue works, in general, on a positive water balance.

(*d*) There must be a constant supply of growth hormones to the growing regions. Auxins promote the growth of cells in a shoot (see section H); and there is evidence that other special substances (see section I) may stimulate the growth of roots and shoots. These substances resemble the vitamins required by growing animals in that they act in very low concentrations, but are at present described as phytohormones because they are produced by the growing plant and are not provided by the environment. The extent to which the activity or dormancy of growing tissue is determined by the presence or absence of hormones is a problem which is under investigation.

Necessary external conditions.[1] I. *For a growing organ containing adequate food reserves* (*e.g.*, *germinating seeds, sprouting underground perennating organs or woody twigs*). (*a*) *Available water* (p. 103) must be present, and the *hydrion concentration* and the *balance of inorganic salts* (p. 86) in the external medium must be suitable. (*b*) The *temperature* must be greater than the minimum temperature below which the particular organ fails to grow, and less than the maximum temperature, *i.e.*, that temperature which sooner or later is injurious to the organ (*cf.* pp. 5 and 282). (*c*) The percentage of *oxygen* must be sufficient to inhibit anaerobic respiration (p. 284). (*d*) Narcotic conditions (*e.g.*, too high a concentration of carbon dioxide) must not exist. Poisonous substances must be absent; thus lichens rarely grow in manufacturing towns, and algæ and many other water plants are killed by copper salts, even in low concentrations. (*e*) Certain organs (*e.g.*, mistletoe seeds) must, under natural conditions, receive *light* before growth will begin. Light, acting as a stimulus, releases the latent powers of growth. Most organs will grow in the dark, but light is essential for the development of a healthy instead of an etiolated shoot system.

II. *For independent seedlings and older plants.* In addition to all the requirements listed in I., other conditions are necessary for the continued growth of plants belonging to this class. (*a*) The *essential elements* (p. 166) must be supplied in a suitable form for absorption,

[1] The discussion of the ecological problem of the growth of different plants in relation to environmental factors is outside the scope of this book. The reader is referred to Lundegårdh (*186*).

i.e., as carbon dioxide, water, and mineral salts.[1] The importance of trace elements, at any rate for certain plants, is recalled. (*b*) Developing shoots require *light* from the blue-violet end of the spectrum for the formation of leaves and of chlorophylls *a* and *b* (light as a formative stimulus, p. 343). Light from the red end of the spectrum (p. 230) is essential in order to provide energy for photosynthesis, and hence for the production of all the organic food stuffs.

G. The Rate of Growth

The rate of growth in length. As may readily be observed by making measurements of the lengths of whole plants or plant members, or of marked zones in growing members, growth in length runs its course in a characteristic way. For all plant members the rate of enlargement is slow at first, and later increases rapidly to attain a maximum velocity.) For example, in one experiment it was found that the stalk of the sporogonium of *Pellia epiphylla* elongated 1–2 mm. in 2–3 months, and then suddenly elongated 80 mm. in 3–4 days. Later the rate steadily diminishes, and, finally, growth ceases. A sigmoid curve (fig. 52) is given by plotting lengths of stem or root, or areas of leaves, against time during the whole duration of what Sachs termed the grand period of growth. It appears that the form of this grand curve of growth is for any member governed by its genetical constitution. External conditions can alter rates, and hence affect the duration of the grand period, but the sigmoid form of the growth curve is always maintained. Stiles (*291*) considers typical instances in chap. XVI of his book.

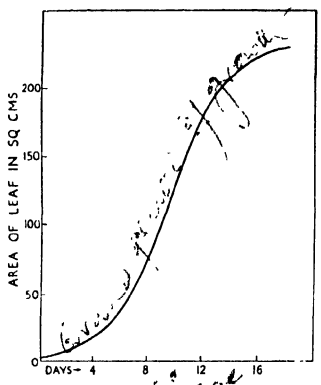


FIG. 52. Sigmoid curve of increase in area of the surface of a leaf of *Cucumis sativus* (from Gregory, *101*).

The rate of growth in length, then, is governed by internal and

[1] Although green plants can grow in media completely free from organic substances, it has been found that minute doses of extracts of dung may greatly stimulate the growth of certain plants (*e.g.*, the duckweeds). The non-essential but stimulating organic dung extracts of unknown chemical composition have been given the name *auximones*. For a summary of recent investigations into possible stimulations by such extracts, animal hormones including sex hormones, pantothenic acid, and other products containing organic substances, see Thomas (*309*). Physiologists have found the evidence conflicting; but some of the reported increases in the field of crop plants have been sufficient to make it not impossible that patent manures containing special substances will appear on the market in the future.

external factors. For any plant or part of a plant, at any time, there is a maximum rate of elongation, determined by inherited genetical factors, which cannot be exceeded, whatever the environmental conditions may be. Much variation in this maximum rate is encountered from plant to plant. Plainly, the average rate for the whole growing period is relatively high in the stems of herbaceous annuals which attain, when fully grown, heights of six feet or more, and relatively low in plants that possess short stems as one of their racial characters. For a given plant or part of a plant, at any time in the grand period, external conditions affect the rate of growth by determining how near to or how far from the inherent maximum rate for that time the actual growth rate will be. Consistently favourable external conditions over a whole growing season, a rare occurrence in nature, would cause the average rate to approach the inherent maximum and relatively tall specimens of the species would be produced. But, however favourable the conditions, the tallest specimens of dwarf races would still be short.[1] Conversely, consistently unfavourable conditions, and these are of more frequent occurrence, would stunt growth; and even potential giants might when fully grown be but puny specimens of their race. For variable external conditions, such as are encountered in a normal growing season, the modes of interplay between the internal factors that determine the form of the grand curve of growth, and the assemblage of significant external factors, are so varied and complex that analysis cannot be attempted here. It must suffice to consider the effect on a given plant over a defined period of varying single factors, one at a time, all the others being kept constant.

We can at once state that the absence of a necessary factor (*e.g.*, one of the essential elements) or the prevalence of an inhibiting factor (*e.g.*, too low or too high a temperature) cannot be compensated for by the presence of all the other factors in highly favourable amounts. The growth rate would sooner or later fall to zero, were the environmental conditions completely unsuitable in one respect only. In a plant which had not suffered permanent injury, growth would be resumed on supplying the missing factor or neutralizing the effects of the inhibiting factor, and the rate attained would in part be determined by the quantitative nature of the treatment adopted. Certain of the responses, such as those of various organs to different concentrations of oxygen, are chiefly of theoretical interest; but others, such as the reactions to different concentrations of mineral salts containing the essential elements, are of great practical importance, for they throw light on the advantages to be gained by mineral

[1] This is simply stating in terms at present in use what Gregor Mendel educed from the results of his breeding experiments on tall and dwarf races of *Pisum sativum*.

manuring at different periods in the growth of crop plants (see Miller, *201*; Russell, *255*; Gregory, *102*).

Increasing the temperature above the minimum has the same effect on growth as on photosynthesis (p. 234) and respiration (p. 282). At first the rate increases (and Q_{10} is often greater than 2), but sooner or later injurious effects bring about retardation, and, at the apparent maximum temperature, growth ceases. For any definite set of conditions there will thus be an optimum temperature. What this is largely depends on the duration of the experiment. Temperatures of 30° C. or more might appear to be very favourable in short duration experiments (*e.g.*, of twelve hours) but deleterious over longer periods.

The importance of a water balance for the promotion of turgor expansion in a growing plant has already been discussed (pp. 103 and 124). It is a matter of common observation that the rate of growth is affected by the water content of the soil, and that the tendency of plants to wilt in dry air is antagonistic to growth.

In the field the rate of growth of a given plant in a soil of approximately constant composition is largely governed by the water supply, the humidity of the air, and the temperature. A. M. Smith observed that the growth rate of bamboo shoots in Ceylon appears to be sometimes determined by the saturation deficit of water vapour in air, and sometimes by the temperature. During the night the air was saturated with water vapour, and the growth rate fluctuated with the temperature. During the day although the temperature rose the growth rate fell more than could be accounted for by the retarding influence of light. This fall appeared to be correlated with an increase in saturation deficit brought about by a decrease in humidity as well as by the rise in temperature. Another good example is provided by the "sunshine-effect" reported by Balls for the cotton plant and other plants growing in Egypt. It appears that direct insolation often immediately checked the elongation of stems. That water loss was the cause of arrested elongation was proved by showing that growth was renewed if the illuminated plants were either defoliated or placed in humid air under bell jars.

Although plants will grow in the dark, light, acting as a formative stimulus and providing energy for photosynthesis, is an essential factor for the healthy growth of green plants. In this section attention is particularly directed to the important fact that light retards the rate of growth in length of stems. The comparison of the lengths of the stems of plants grown in darkness (etiolated plants) and those of control plants grown under normal conditions establishes this fact. Further, for short experimental periods, the auxanometer (fig. 53) may be used to show that when all conditions other than light intensity are kept constant, an inverse relationship exists between the

growth rate of a stem and the intensity of daylight. Thus in the given record of an experiment, it will be seen that the marks made during the day are closer together than those made during the night. We must therefore recognize a daily periodicity, governed by light intensity, in the growth in length of shoots. The retardation brought about by light in the daytime may have certain functional advantages. Thus the rate of turgor expansion may be adjusted by light to the rates at which the enlarging cells and differentiating mechanical tissue can use foodstuffs and so consolidate growth. According to this view the nice proportions of a healthy plant may owe much to the retarding effect of light on growth, which in consequence is often spoken of as a regulatory effect.[1]

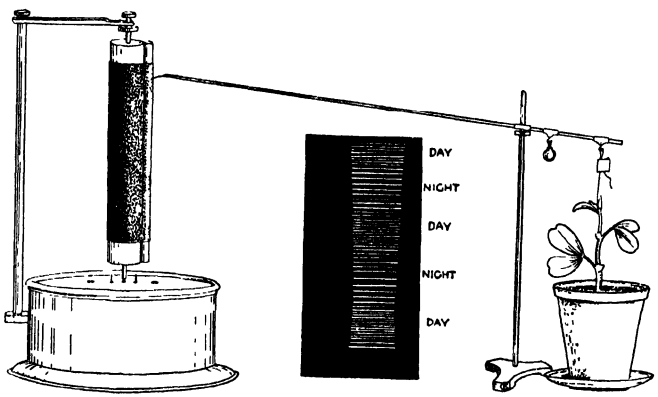


FIG. 53. The auxanometer for measuring the relative rates of growth of a given plant member under different environmental conditions. Growth increments are magnified by means of a lever arrangement, and a record is made on the rib of a smoked drum, which is clock-driven about a vertical axis.

The quantitative study of light-growth reactions and of the part played by auxins therein.[2] The quantitative work initiated by Blaauw was later extended, especially in Went's laboratory at Utrecht,[3] and led to additions in our knowledge of the responses of plants to light. The pioneer work in the last century had been on the effects induced by different *intensities* of light on the growth rate. The duration of the period of illumination was ignored. Blaauw

[1] Bibliographies concerning important advances in our knowledge of the effects of varying amounts of light on growth and development have been given by other authors (*e.g.*, Barton-Wright, *17*, Miller, *201*, Hamner, *113*, Whyte, *334*). We have no room here to discuss this subject of photoperiodism, which has considerable practical applications.

[2] Section A in chap. XVII should be read before this subsection.

[3] See Professor Went's review in Kostytschew's book (*174*), and the summary of a presidential address (*324*).

made the fundamental advance of considering time as a factor in studying light-growth responses., In his experiments he measured the growth responses of certain plant organs to different *quantities* of light, as measured by the product of intensity (expressed in metre candles) and duration (expressed in seconds). Other workers have since used the same unit, the metre-candle-second (m.c.s.).

With a view to obtaining significant quantitative data, special attention has been paid in all the recent experiments to the selection of plant material and control of experimental conditions. Wherever possible pure lines have been used, fair samples subjected to experiment, and due allowance has been made for experimental and sampling errors. The data have been gathered by ingenious and precise experimental methods. In the experiments performed at Utrecht, the plants to be investigated were reared in the dark (or in red light, since it has been found that light of this colour has practically no effect on the growth rate) in a chamber or room kept under constant conditions of temperature and humidity. Growth was measured by the horizontal microscope or by a specially designed modern form of auxanometer, in which the relative times taken to make a definite growth increment are recorded on a smoked drum outside the growth chamber. Growth has also been cinematographed, using panchromatic plates, and its progress and rate studied from the positives of the films.

After a period of steady growth in the dark or in red light, a definite quantity of white light, measured in metre-candle-seconds, was allowed to fall on the organ from above. In order to avoid phototropic stimulation the growing organ was placed from the beginning of a period of illumination between mirrors arranged so as to ensure equal illumination from all sides. A definite intensity of light for a single short period of illumination (*e.g.*, 100 metre-candles for five seconds) was used, and when the period was over, the subsequent growth rate of the organ in the dark was measured. Using this method, Blaauw, and later the Utrecht workers, measured the growth responses of various plant organs to different quantities of light. So delicate were their methods of measuring growth that responses were observed less than five minutes after illumination.

In these experiments the problem of light-growth response was reduced to its simplest form, and one in which the exact quantities of a single stimulus and response could be measured. This is the proper scientific approach to the much more complex problem of interpreting the total effects produced under natural conditions of alternating long periods of day (with varying light intensities) and night. The solution of this complex problem is, of course, the ultimate objective. The first few steps along the right road have been taken.

Blaauw studied the light-growth responses of sporangiophores of

Phycomyces nitens, the hypocotyls of sunflower seedlings, and the roots of white mustard, oat, and radish. The first after-effect of illuminating the sporangiophore of the mould fungus with quantities of light varying from 1 m.c.s. to 4,000 m.c.s. was an acceleration of growth. This was observed after about six minutes, and was followed by a succession of retardations and accelerations until the light-growth response was over (fig. 54). The quantity and form of response varied with the quantity of light used. Since for each quantity of light the sporangiophore grew more rapidly than if it had been kept all the time in the dark, light in these experiments may be said to have promoted the growth of the sporangiophore. On the other hand, the total resultant effect on the hypocotyl of sunflower seedlings of light quantities under 4,000 m.c.s. was found to be a retardation. With

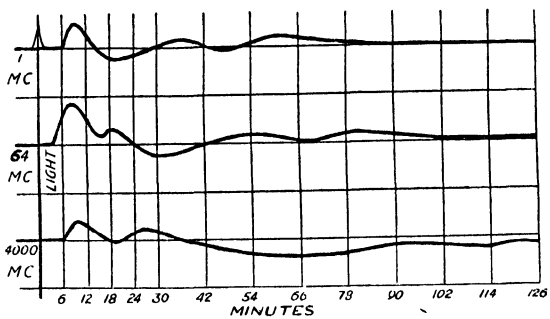


FIG. 54. The light-growth reaction of the sporangiophore of Phycomyces in response to uniform illuminations of 1, 64, and 4,000, metre-candles, given at the instant indicated by the arrow. (From Blaauw, see Kostytschew, *174*.)

quantities of light greater than 4,000 m.c.s. the behaviour of the sporangiophore became qualitatively as well as quantitatively different. The positive response of accelerated growth was succeeded by indifference, a negative response, and a second positive response, for increasing quantities of light.

The roots of oat and radish showed no light-growth response ; but the roots of white mustard responded, and with the quantities of light used in the experiment growth was promoted. The discovery that roots of different species may behave differently towards light has an important bearing on theories of phototropism (p. 404).

Several workers have investigated the growth responses of the coleoptile of the oat to different quantities of light. Arisz reported that the total effect was a retardation for quantities of light under 4,000 m.c.s. For higher quantities positive reactions (accelerated growth rates) were observed, and retardations again set in with quantities of light greater than 70,000 m.c.s. Van Dilleweyn later

extended this work, and used his own and Arisz's experimental results in his analyses of the various phototropic curvatures shown by the oat coleoptile (p. 404). For 500 m.c.s. F. W. Went confirmed Arisz's finding when he observed that for the whole organ the total effect was a retardation (fig. 55). He discovered that this total response was composed of at least two reactions, namely, that of the tip and that of the base. The tip reaction was evoked by illuminating this part only, and, although it set in later, the reaction was stronger than that produced by illuminating only the base. In the tip reaction, a strong retardation was observed after twenty to thirty minutes. This was followed by a slight acceleration, and the whole reaction ended after

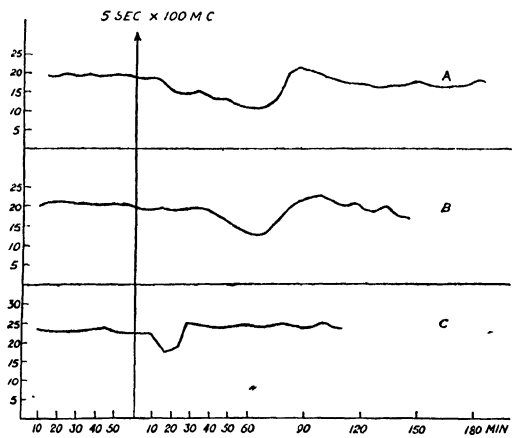


FIG. 55. The light-growth reaction of the coleoptile of oat in response to uniform illumination of 500 metre-candle-seconds, given at the instant indicated by the arrow. (From F. W. Went, see Kostytschew, *174*.) A. The response when the whole coleoptile was illuminated. B. The response when the tip alone was illuminated. C. The response when the base alone was illuminated.

two to three hours. The base reaction began much earlier, and consisted of a marked retardation which was followed by a slight acceleration and then the reaction ceased.

It appears therefore that light, in addition to having a direct effect on regions of enlargement (base reaction), acts indirectly through affecting the influence the tip exerts on growth in length. This suggested to Went that the primary effect of light in the tip reaction might be upon the amount of auxin in the tip. Accordingly, he measured the auxin content (for method see p. 369) of the tips of oat coleoptiles which had grown in absolute darkness, and of others which had received 1,000 m.c.s. of light from above. He found that the latter when placed eccentrically on decapitated stumps caused twenty per cent. less divergence from the vertical than did the tips which

had received no light. He inferred that 1,000 m.c.s. had destroyed or annulled twenty per cent. of the auxin, and attributed the light-growth retardation produced by illuminating the tip of this organ to the diminished downward flow of auxin from the tip to the cells in the enlarging region.

Van Overbeek (*221*) investigated the influence of light on the base reaction of oat coleoptiles to auxin-a and to heteroauxin. In order to prevent the regeneration of auxin-a he used twice decapitated coleoptiles, and topped them one-sidedly with agar blocks containing phytohormone. Curvatures away from the covered sides occurred both in the dark and when the stumps were uniformly illuminated; but whereas in the experiments with auxin-a the curvatures were less in the light than in the dark, those shown in the experiments with heteroauxin were hardly diminished at all by the action of light. These results clearly suggest that auxin-a is liable to undergo photo-inactivation. This is probably an oxidative process. In contrast the photostability of heteroauxin is noteworthy. V. J. Koningsberger and Verkaaik (*173*) point out that evidence has been obtained in Kögl's school that, when in solution *in vitro*, auxin-a exists in equilibrium with its lactone, which also possesses growth-promoting properties; but that whereas auxin-a is photostable, auxin-a lactone is photolabile, being converted by ultraviolet light into inactive lumi-auxin-a lactone. Koningsberger and Verkaaik suggest that a similar inactivation occurs in the coleoptile in blue-violet light (4,900–3,700 Å), which is known to inhibit strongly the growth of coleoptiles. C. Koningsberger has shown that light of this quality is not absorbed by auxin-a, but it is well known that carotinoids, which are always present in coleoptiles, show strong absorption bands in the blue-violet region of the spectrum (p. 230). The inference drawn is that in reacting cells exposed under normal conditions carotinoids may act as sensitizers for the photochemical inactivation.

Apparently the annulling of auxin by light is not a general phenomenon. Van Overbeek (*220*) found for Raphanus seedlings that the auxin content of the cotyledons and the tip of the hypocotyl did not diminish when they were illuminated. His experiments indicated that the diminished growth of the hypocotyl in the light results from the reduction light induces in the sensitivity of enlarging cells to auxin. He placed agar blocks one-sidedly on hypocotyls from which the cotyledons had been removed, and subsequently left some in the dark and some in the light. He found that curvature in the dark was on the average nearly three times as great as in the light. He realized that this might have been due to the retarding influence light exerts on the transport of auxin on the illuminated side of the hypocotyl, for the elongating cells on the dark side would then receive relatively more auxin, and curvature would inevitably follow. To

test this possibility he set up a system composed of a cylindrical piece of hypocotyl with an agar block containing auxin fixed on its top surface and a receiving block of agar at its base, and measured by Went's method the amount of auxin that collected in the lower block when the hypocotyl was in the dark and when it was illuminated. His results indicated that "the quantities of growth substance transported through hypocotyls in the light certainly are not less than those transported in the dark". Hence he attributed the curvatures with one-sided illumination to the fact that "in the dark the cells are more sensitive to growth substance than in the light".

Finally it must be pointed out that light may have effects on enlarging cells that are quite independent of the relations of these cells

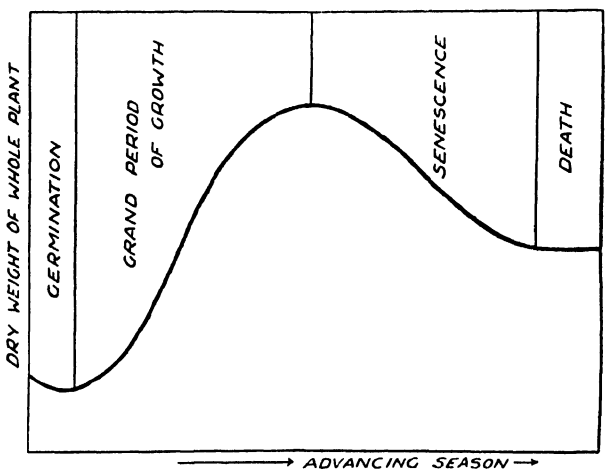


FIG. 56. Generalized form of growth curve showing changes in dry weight which occur during growth and senescence, and after death.

to auxin. Van Dilleweyn suggested, when considering the base reaction of the oat coleoptile, that light may affect growth by inducing changes in the permeability of protoplasm in the enlarging cells.

The rate of increase in dry weight. It has long been known that changes of dry weight accompany the growth of plants. Indeed, when dry weight is used as an index of growth, a grand period can be recognized and a grand curve of growth constructed (fig. 56). Loss of dry weight occurs during certain periods (see below), but, in general, the curve takes a sigmoid form until the plant is fully grown. During senescence the dry weight steadily decreases, and at death a skeleton composed of cell walls and certain residual solid cell contents is left. When the dry weight begins to increase, the *rate of increase* rises to a maximum, and then, in general, declines (fig. 57).

Photosynthesis is responsible for the formation of more than ninety per cent. of the dry matter of plants. The remaining dry matter results from the absorption of mineral salts, and, to a lesser extent, from certain intramolecular changes such as hydrolyses. Slight decreases in dry weight are caused by condensations that occur with the elimination of water, but loss of dry matter results chiefly from respiration. Plainly, the difference between the rate of photosynthesis and the rate of respiration is the principal factor which, at any time, determines the rate of change of dry weight. It follows as a corollary that a steady loss in dry weight occurs when photosynthesis is not in progress (*e.g.*, before green leaves unfold in the germination of seeds or the sprouting of buds, in defoliated perennating organs, and during the night in growing plants possessing green leaves).

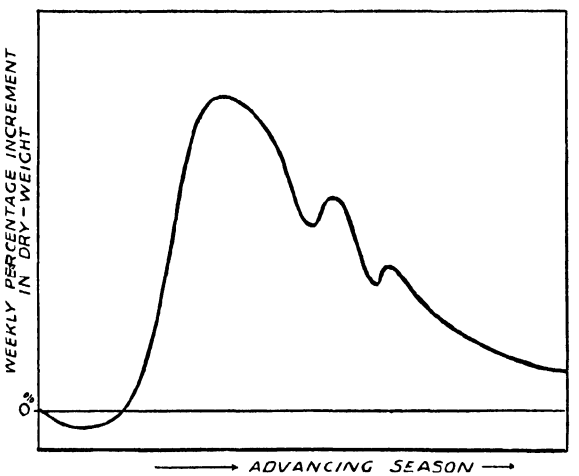


FIG. 57. Generalized form of growth rate curve of maize. (From Briggs, Kidd, and West, *56*).

Much variation will be encountered in a given plant, according to the stage of development of the plant, since, under constant external conditions, the rate of photosynthesis will be governed by the number of green leaves and the activity of their constituent chloroplasts, and the rate of respiration by the number of living cells and their average respiratory activity. Further, it is clear that external conditions affecting the rate of photosynthesis and respiration will also affect the rate of growth. Clearly, therefore, in the study of the subject of plant yield due attention must be paid to the inherent internal factors and to the effect of external conditions. The subject is so important that it is not surprising that a large mass of quantitative data has been gathered for crop plants (see Russell, *255*).

The possibility of subjecting the results of experiments on growth rates to mathematical treatment has attracted many workers and such attempts as have been made have added considerably to our understanding of the factors which govern dry weight increase. Slator has succeeded in showing that the growth of yeasts and of other unicellular organisms, when measured by dry weight changes, appears to obey a compound interest law. In such organisms, however, all the cells formed during growth retain the power of assimilating foods. Productive matter is continuously forming more productive matter, and it appears that for any period the rate of dry weight increase per unit initial dry weight is constant.[1]

Simple mathematical treatment by the compound interest law is not, as a rule, possible for the higher plants. It is true that through the first half of the grand period of growth the rate of increase of dry weight steadily increases with time. This, of course, is what one would expect, seeing that during development total photosynthesis steadily increases, owing to the formation of additional green leaves. But non-productive dead tissue (*e.g.*, wood vessels and fibres) and actively respiring non-green vacuolated parenchyma are also formed. Consequently there is a continuous heaping up of non-productive dry matter, and consumption of respirable substrate; and thus for successive equal periods of time the ratio of the dry weight at the end of the period to the initial dry weight steadily falls, instead of remaining a constant as the compound-interest law would require. For crop

[1] Thus having experimentally found the percentage rate of increase of dry weight for a given race of yeast for defined external conditions, we may calculate and so predict what the final dry weight M will be if the initial mass M_0 is allowed to grow for x units of time. The compound interest law requires that for a rate of r per cent..

$$M = M_0\left(1 + \frac{r}{100}\right)^x.$$

This relation is often expressed in another form.
Since

$$\left(1 + \frac{r}{100}\right)^x = e^{x\log_e}\left(1 + \frac{r}{100}\right),$$

we may write

$$M = M_0 e^{x\log_e}\left(1 + \frac{r}{100}\right),$$

or

$$M = M_0 e^{ax}$$

where the symbol a is used for

$$\log_e\left(1 + \frac{r}{100}\right).$$

The rate of increase at any time is then readily calculated, for if

$$M = M_0 e^{ax},$$

the rate after x units of time will be given by differentiation and

$$\frac{dM}{dx} = M_0 a e^{ax}.$$

plants, then, it is not surprising that the expression in mathematical form of such tentative generalizations as have been arrived at has proved a difficult and controversial matter (see Barton-Wright (*17*)).

For such higher plants as consist mainly of green leaves (*i.e.*, of productive assimilating tissue) relatively simple quantitative relationships have been worked out from the experimental results. It has been reported for maize that the ratio of the rate of dry weight increase to the leaf area is approximately a constant during the growing period. And the work on *Lemna minor* by Ashby (*6*) and others in V. H. Blackman's laboratory indicated that, for any given set of conditions, the curves of increase in frond number, frond area, and dry weight, plotted against time, approximate to an exponential type (such as a compound interest law would require) of which the numerical constants may be calculated. These floating water plants were allowed to multiply by vegetative propagation under rigidly controlled conditions, which could be varied so as to determine the influence of external factors on the growth constants. Hicks (*128*) thus found that Q_{10} for growth was 2·8, and that the optimum growth rate occurred at 30° C. when a constant light intensity of 1,000 foot-candles was used. The fact that figures such as these were quoted with considerable reliance on their significance testifies to the clearer understanding physiologists have been acquiring of growth problems, and to the improvement in the experimental methods used in the study of these problems.

CHAPTER XVII

PLANT GROWTH SUBSTANCES[1]

In this chapter we shall describe the activities of organic compounds, other than the recognized foods, which must be supplied to a cell before it will show growth processes such as those described on pp. 341 to 352. This definition implies that *growth substances* affect in some essential manner one or more of those varied physical and chemical events which occur during meristematic activity, cell enlargement, or cell differentiation. Furthermore, the compounds have, like the so-called micronutrients containing trace elements, the physiological property of exerting their function in very low concentration. If we place a compound having such a property in a class called *trace substances*, we may conveniently distinguish between organic trace substances, some of which (viz., those essential for normal life) we shall consider in this chapter, and the inorganic trace substances mentioned on pp. 168–9.

When a substance is supplied to a cell it must obviously come from outside the cell. A growth substance supplied by the external environment of an organism is called a *vitamin* if it controls the growth of an animal, and a *bios factor* if yeast will not grow without it. We exclude from the genus growth substance, although not from the class of trace substances, organic compounds (*e.g.*, auximones) which may stimulate but are not essential for growth.

A substance essential for a growth process may, however, be supplied to a developing tissue, not by the environment outside the organism, but by another tissue within the same organism, where it is synthesized. The term hormone was first used by Starling in 1906 in describing the action of secretin in the animal body, and he later defined a hormone as " any substance normally produced in the cells of some part of the body and carried by the blood stream to distant parts which it affects for the good of the body as a whole." We may widen the extension of the term and use it for multicellular organisms without a blood stream. Modifying slightly a definition made by J. S. Huxley, we shall define a *phytohormone* as a migratory trace

[1] Authoritative monographs describing the earlier work on plant hormones, and certain other growth substances, with extensive bibliographies, have been written by Boysen-Jensen (*45*) and by Went and Thimann (*328*). The reviews *Über Wuchsstoffe* written by Jost (*159*) are very interesting in that they reveal the impressions that the discoveries of the 1920's and 1930's, and the suggestions concerning the interpretation of the discovered facts (see, *e.g.*, footnote on p. 4), made upon the mind of an eminent senior physiologist. On p. 158 of an article by Robbins and Schmidt (*249*) some of the advantages attaching to the continued use for the present by botanists of the general term growth substance are clearly stated.

substance which is produced in one tissue of a plant, and, later, exerts a specific effect of functional value on another tissue in the same plant. Accordingly those plant growth substances which migrate from a region of synthesis to the growing regions where they exert their specific influences may be described as phytohormones. They are sometimes called growth hormones to distinguish them from other phytohormones which are concerned with processes distinct from growth, for example, with pulvinar movements of variation (p. 411).

Many years ago Sachs, Beyerinck, and Jost suggested that migratory growth substances occur in plants (see pp. 349 and 375). We now possess proof of their existence. They may be regarded as *correlating substances* or *correlators*, which play an essential part in the integration of plant behaviour (p. 348).

A. Auxins as Phytohormones that Regulate Growth

The discovery of growth-promoting substances (auxins) in shoots. During the last thirty years remarkable researches have brought to light the fact that, in addition to foodstuffs and water, some other substance is essential for the enlargement of newly formed cells. The story begins with Boysen-Jensen's discovery that whereas, as Rothert had earlier reported (p. 397), a coleoptile of oat (Avena) loses its power of responding to a phototropic stimulus when its tip is cut off (1–2 mm. being removed by a clean transverse cut), this power is restored by replacing the cut-off tip directly or fixing it with gelatin on the headless stump. The phototropic stimulus did not pass when tip and stump were separated by a mica plate. The evidence clearly suggested that a diffusible substance was involved in the basipetal transmission of the phototropic stimulus from the region of perception (the coleoptile tip) to the growing region, where curvature was seen. In general, Paál confirmed Boysen-Jensen's findings, showing that cut-off tips, but not bits of stump, restored the power of responding to the phototropic stimulus. Since the stimulus did not pass across platinum foil, he inferred that it was material and not electrical, and its passage through gelatin, but not through cocoa butter, suggested that it is a water-soluble diffusible material.

Paál's major contribution to knowledge, however, was his demonstration that the diffusible substances that correlate the activities of tip and stump control the growth rate not only when phototropic curvatures are taking place, but also during normal straight growth. He experimented with seedlings of the oriental grass, *Coix lachryma*, in which the coleoptile is borne aloft on a straight hypocotyl. He cut off this coleoptile, and then, by means of gelatin, fixed it eccentrically on the cut surface of the hypocotyl. Curvatures occurred both in the dark and when the seedling was uniformly illuminated, and always away from the covered side. Moreover he found that the rate of

straight growth of the hypocotyl was strongly retarded by cutting off the coleoptile, but nearly attained its normal value again when the coleoptile was fixed centrally in its proper position on the stump. His general conclusions were that substances, which he described as correlation carriers, are always present in the coleoptile tips of graminaceous seedlings, and that they promote growth in length of the coleoptile and hypocotyl. Straight growth occurs when they diffuse morphologically downwards at equal rates on all sides. The

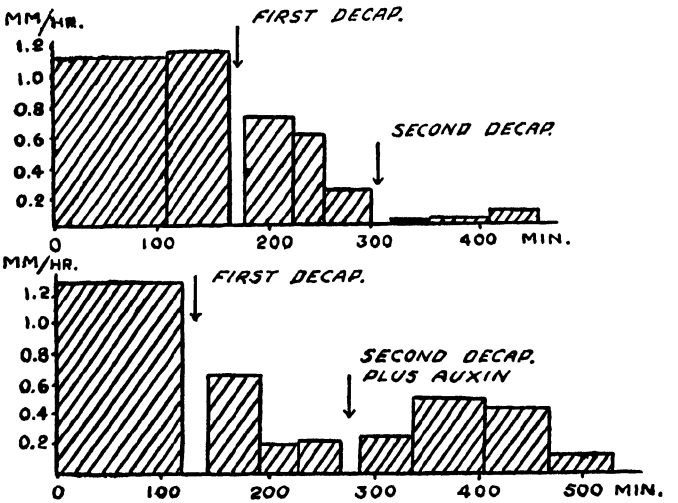


FIG. 58. Growth rates of Avena coleoptiles in millimetres per hour, as determined by Dolk. The upper figure illustrates the effect of two decapitations in permanently depressing the growth rate almost to zero. The lower figure records the resumption of growth when auxin is applied after the second decapitation. (From Went and Thimann, *328*, p. 74.)

evidence indicated that migration is essentially in a longitudinal direction ; for some reason lateral transport of the substances is very slow. Accordingly, when coleoptiles were fixed eccentrically on *Coix* hypocotyls, the growth-promoting substances attained a higher concentration on the covered side of the enlarging regions. Consequently curvature occurred away from the covered side.

The gradual growth of knowledge and the development of experimental methods are described in the monographs cited on p. 364. The work done in several continental centres prepared the way for the fundamental researches on what we now call auxins, that were carried out later at Utrecht; quantitative physiological researches in the laboratory of the late Professor F. A. F. C. Went, and chemical researches in Professor Kögl's laboratory. Thus certain workers (*e.g.*,

Stark, Seubert) studied effects induced in headless coleoptile stumps by agar blocks containing plant extracts or other products (*e.g.*, saliva), which had been dissolved in these blocks. Söding measured the straight growth of coleoptiles in the dark, observing that for five hours after decapitation growth was retarded. Growth did not stop, probably owing to the presence of residual auxin in the stumps. Subsequently an acceleration of growth set in. This he attributed to the regeneration of growth substances at the head of each stump, which then acted as a new " physiological tip." At a later date Dolk (see fig. 58) found that a second decapitation made two hours after the first brought growth practically to a standstill.

The experiments at Utrecht on the growth of coleoptiles were carried out in the dark or in red light (see p. 356) in rooms kept at constant temperature and constant humidity. A pure line of Avena was used, and samples were made sufficiently large to ensure that conclusions would only be drawn from significant differences. F. W. Went completed the proof that growth-promoting substances are present in coleoptiles, by extracting them and measuring their effect on growth.

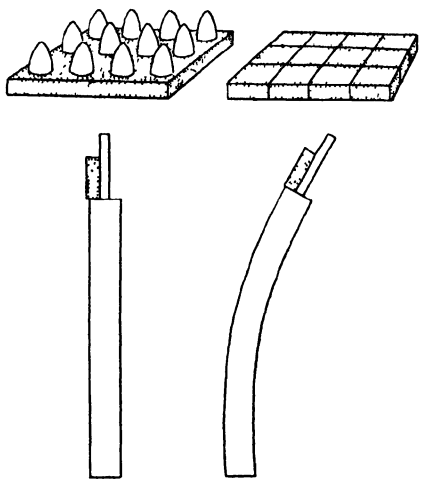


FIG. 59. F. W. Went's method for extracting and experimenting with auxin (see Kostytschew, *174*). 1. Twelve coleoptile tips are placed on an agar plate. 2. The agar plate is subsequently cut into twelve blocks. 3. One block is placed eccentrically on the stump of a decapitated coleoptile. 4. Curvature of this coleoptile away from the covered side.

To extract the auxin he cut off the tips of oat coleoptiles and placed them on a plate of three per cent. agar. To demonstrate that auxin had actually diffused out of the tips into the agar, he removed all the tips after one hour, cut the agar plate into small blocks of equal size, and then placed some of the agar blocks centrally and others eccentrically upon decapitated coleoptiles (fig. 59). Growth was in both instances promoted. The graphic record (fig. 49) of a later experiment of Dolk's on twice decapitated coleoptiles illustrates this growth-promoting action. Went concluded from his experiment that auxin must have passed first out of the tips into the agar and then out of this gel into the decapitated stump. In the stump the auxin must have been translocated basally to the elongating regions, where it

would enhance turgor expansion. Stumps covered with centrally placed blocks grew vertically. Apparently the auxin was uniformly distributed all round the elongating coleoptile. With the eccentrically placed blocks, however, relatively more auxin must have arrived at the elongating cells on the side covered by the block than on the other side, for growth was more rapid on the covered side, and the coleoptile curved away from this side. It has since been shown that the angular divergence from the vertical is in such experiments determined by the concentration of auxin in the agar block, until a maximum concentration is reached above which there is no increase in curvature. Went believes that until this maximum is reached the concentration of auxin limits the rate of growth, but that with higher values the amount of food material for cell growth becomes the limiting factor. In high concentrations auxins may inhibit the growth of members of a shoot system (see Thimann, *299*).

It should be noted that Went successfully repeated Paál's experiments, using not the living tissue itself, but auxin contained in extracts from living cells, as a source of growth-promoting substance. The problem of isolating pure auxins from these extracts or from other sources was defined. As we shall point out shortly this problem has been solved. Consequently Paál's and Went's experiments can now be performed with seedlings and pure chemicals; and, for a given population and specified conditions, predictions that are approximately fulfilled can be made concerning the quantitative results that will be obtained.

Auxins and the growth in length of roots. The results of the investigations of Cholodny (*60*) on the action of growth stuffs on roots have been confirmed by Keeble, Nelson and Snow (*161*). It appears that although the growth rate of roots is affected by auxins, these organs respond differently from stems. Thus maize roots continue to elongate after decapitation (*cf.* the behaviour of the oat coleoptile, p. 365), and re-heading with root tips leads not to an acceleration of growth, but to a retardation. Cholodny found that re-heading with coleoptile tips had the same effect, and inferred that root tips produce the same growth regulator (now called auxin) that governs the growth of stems, and that it migrates from the root tips to the elongating regions. He supported this view by showing that root tips can accelerate the growth of decapitated coleoptiles. He concluded that it is not the nature of the substance but the behaviour of the elongating cells in the presence of the substance that accounts for the contrary behaviour in roots, *i.e.*, that whereas the stimulus is simple and chemical the response is complex and biological.

Using F. W. Went's method, Keeble, Nelson and Snow (*loc. cit.*) and Hawker (*120*), made experiments with agar blocks and obtained results in accord with Cholodny's. For instance, agar blocks

containing auxin from root tips of maize or of the broad bean, when placed centrally on their respective roots, retarded the growth rate and there was no curvature. When the blocks were placed eccentrically on the decapitated roots, the re-headed roots curved in the direction of the side which was covered. This can be explained by supposing that relatively more auxin passed to the elongating cells on the covered side, and that the retardation on this side was in consequence greater.

More recent work suggests that auxins in extremely low concentrations may accelerate root growth, *i.e.*, root response may be similar to that of a stem in that it increases up to a certain optimum auxin concentration, and falls off when this value is exceeded, inhibitory effects being shown at auxin concentrations that strongly promote stem growth. Differences in the values of the optimum concentration for stem and root help to account for some of the differences in behaviour these members have shown under experimental conditions (see Thimann, *299*).

The extraction, detection, and estimation of free auxins. Went's diffusion method for extracting auxins has already been described. Extraction with water from crushed tissues has been attempted, but activity is lost through the enzymic oxidation of auxins. Thimann developed a method that overcame this difficulty. The plant tissue was killed by immersing it in a certain volume of chloroform ; one-fifth of this volume of normal hydrochloric acid was then added, and the killed tissue was thoroughly ground ; the chloroform layer was separated off, and the ground acidified tissue was extracted twice more with chloroform ; the lipoidal residue obtained by evaporating off the chloroform contained the auxins that were present in the original plant material. Thimann extracted this residue with 0·3 c.c. water, and the extract was added to 0·3 c.c. of three per cent. agar. Avena coleoptiles were then used for the biological assay of the auxins present. Van Overbeek (*222*) refers to more modern methods for extracting such auxins as may exist in an active state at any time in a given organ, separately from precursors. These auxinogenic substances may greatly preponderate in amount over the free auxins.

We recall that F. W. Went discovered that, up to a certain optimum value, the concentration of auxin in an agar block determines the angular curvature induced in a coleoptile stump, when the block is placed eccentrically on the stump. This discovery suggested a method for estimating the auxin strength of a block, and consequently of the material from which the auxin had been obtained. The unit of representation used by Kögl and Haagen-Smit was the Avena-unit (A.E., which stands for Avena Einheit). 1 A.E. is the amount of auxin in a 2 c.mm. agar block which imparts a curvature of 10° to a decapitated coleoptile of Avena upon which the block is placed eccentrically under the conditions of Went's experiment. The strength of auxin in coleoptile tips worked out at about 300 A.E. per mg. of tip substance, *i.e.*, the auxin in 1 mg. of tip substance (extracted by Went's or Thimann's method) could be made to impart 10° curvature to 300 decapitated coleoptiles. Since the A.E. value of 1 mg. equivalent of heteroauxin is known, the auxin value of any substance or extract under investigation can also be, and often is at present, expressed in terms of mg. equivalents of β-indolylacetic acid (see van Overbeek, *loc. cit.*).

Using a similar test method to Went's, Söding has found that seedlings of Cephalaria are much more sensitive to low concentrations of auxin than are Avena seedlings, and that good results are given in diffuse light as well as in the dark. The use of more delicate methods has established beyond doubt that auxins are present in most regions of growing green plants. Skoog increased the sensitivity of the Avena method by de-seeding the test plants about thirty hours after planting.

Schneider (*262*) has modified Bonner's section test method, which makes smaller demands upon technical skill than does Went's method of assay. Small pieces are cut from coleoptiles, and comparable samples, mounted on the teeth of a fine comb, are submerged in a nutrient solution of one per cent. cane sugar. The underlying principle is to compare the acceleration in the growth rate effected by adding the substance under investigation with that produced by known amounts of heteroauxin.

Certain simple methods are available for class experiments. Auxins dissolve in lanolin, and the solution can penetrate plant cuticle. Such auxin paste, when smeared on one side of a stem, root, or leaf, may induce bending of the organ owing to the unequal growth of opposite sides. The normal responses of organs will be illustrated if for leaves and stems the curvatures are away from, and for roots in the direction of the smeared side; but with high concentrations of auxin abnormal results may be obtained. In one experiment with the stem of the sweet pea, 0·005 per cent. β-indolylacetic acid (heteroauxin) gave good results, as also did 0·25 per cent. phenylacetic acid, a much cheaper synthetic substitute. It is also instructive to compare the growth of decapitated coleoptiles of Avena, some of which have been topped with auxin-free lanolin and others with lanolin containing an auxin. The inhibition of root growth by solutions containing heteroauxin is easily demonstrated. Lane observed that eighty per cent. inhibition of root growth resulted from soaking Avena seeds for one day in water and for another in a solution containing less than 0·004 mg. heteroauxin per cubic centimetre.

The occurrence and production of auxins and auxinogenic substances. An important stage in the development of knowledge was the detection of auxins in a wide range of plants and of tissues in a given plant. It now appears to be probable that auxins are present in all growing organs (*e.g.*, those of germinating seeds, seedlings, developing shoots of forest trees) and in some organs capable of growth (*e.g.*, resting seeds, pollen grains). In certain plants (*e.g.*, oat seedlings, see below) graded differences of concentration have been found, suggesting polar travel from regions of production to regions of growth. The absence of specificity is noteworthy; the same reactions occur in test plants whatever the sources of auxins may be.

Evidence exists that, in addition to such auxin as may be present in resting seeds, there is stored a precursor which changes into auxin when the seeds absorb water. This change occurs either in the light or in the dark. It has been suggested that the precursor gives rise to auxin predominantly in apical regions. Thimann found auxin in all parts of germinating oat seedlings; but the relative concentration of the auxin fell from 0·69 at the coleoptile tip to 0·19 at the base, and then increased to 0·43 at the root tip. Skoog placed an agar block

on a decapitated coleoptile attached to a germinating oat grain. After a period he tested the agar block for auxin, but found none. He reported, however, that the blocks caused a delayed action curvature by coleoptile stumps, and attributed this to the influence of an auxin produced at the cut surface from an auxin precursor that had been trapped in the agar block. He inferred that in the germination of whole grains the precursor travels from the storage tissues to the apical parts of growing members, where free auxin is liberated. From these parts the free auxin may migrate to the growing regions of shoot and root, promoting the growth of shoot members and retarding that of the root. It is possible that all storage tissues contain auxinogens in addition to the recognized foodstuffs, and that these precursors are mobilized prior to the active growth of embryos or resting buds, and move to regions where they are converted into active auxins.

The discovery that in green plants there may be several kinds of active auxins and auxinogens (see van Overbeek, *222*) has ushered in the confused phase in the development of auxinology. It may be that the term auxin has been used ambiguously in the past and that there are, in consequence, fallacies in some of the arguments put forward by pioneers in this field of study.

The active auxins are auxin-a, heteroauxin, and, possibly, certain protein complexes containing active groups. They may all occur together in the same cell. The evidence is good that one of the auxinogens present in storage tissue is an ester. An auxin is produced by the acid hydrolysis of the precursor in maize oil. There is a non-proteinaceous substance in the endosperm of cereals and coconuts which yields an auxin when hydrolyzed by alkalies or lipase. On the other hand, in green cells in general (*e.g.*, in those of spinach leaves and Ulva), and in coleoptile tips, there is an inactive proteinaceous precursor, which, under the influence of a proteolytic enzyme, gives rise to an active auxin. Analysis has indicated that in most tissues only three to ten per cent. of the potentially available auxin present exists, at any time, as active auxin. The remainder is present as auxinogens of various kinds.

Nothing conclusive appears to be known about the metabolism that leads to the synthesis of auxinogens in leaves. These precursors presumably give rise to at least a fraction of those active auxins which promote the growth of independent seedlings and sprouting buds when the auxinogen reserve in storage tissues has been expended. We note that the active auxins coming from the leaves may be chemically different from those set free from precursors supplied by storage tissues. The metabolism leading to the production of stored precursors (*e.g.*, ester auxinogens) in seeds and other storage organs may, therefore, be different from that causing the synthesis of auxinogens (*e.g.*, proteinaceous precursors) in leaves.

It is clear that new problems are being defined. Thus evidence is required concerning the accumulation of ester precursor in developing endosperm or cotyledons. We do not know whether ester formation is at the expense of such auxin of one kind or another as migrates from green shoots to the developing ovules, or whether the whole ester is synthesized in the ovules from sugar or from some other substance which is translocated thereto.

The chemistry of auxins. F. W. Went's researches pointed the way to the chemical investigation of Kögl and his collaborators, who have discovered that three distinct auxins are present in human urine, viz., *auxin-a* (a hydroxy acid, $C_{18}H_{32}O_5$), *auxin-b* (a keto acid, $C_{18}H_{30}O_4$) and *heteroauxin*, which is β-indolylacetic acid, $C_{10}H_9O_2N$, and has been synthesized. Auxins a and b have been obtained from certain crude vegetable fatty oils, and also from malt. There is some evidence that auxin-a is present in the coleoptile tips of cereal seedlings, but the presence of auxin-b is not excluded. These auxins are taken in by animals with their vegetable food and pass unchanged through the body. Heteroauxin probably owes its origin to the decomposition of proteins containing tryptophane, *e.g.*, by bacterial action in the digestive tracts of animals, and by the metabolism of yeast and mould fungi growing on culture media. It probably occurs in green plants. It is remarkable that substances so very different in chemical structure as are heteroauxin on the one hand and auxins a and b on the other should exert similar effects on the higher plants. Moreover, similar growth-promoting properties are possessed, but to a less extent, by a number of synthetic organic compounds, *e.g.*, phenylacetic acid, but it has not so far proved possible to assign growth-promoting powers to specific intramolecular structures.

Translocation of auxins. Van der Weij's experiments (see Snow, *275*) have clearly shown that the phenomena occurring during the translocation of auxin cannot be completely explained by the laws of diffusion. Apparently the structure of the growing member and the properties of living cells play a part. For example, it was inferred that movement of auxin can only take place in the morphologically downward direction when it was found that if a short length of coleoptile is placed between two agar blocks, one containing auxin and one without, auxin is appreciably translocated only when the block containing it is affixed to the morphologically upper side of the piece of coleoptile. Furthermore, there is evidence that under these conditions the rate of movement of auxin is greater than can be accounted for by simple diffusion (*cf.* translocation of solutes, p. 149). It also appears that translocation will continue in the morphologically downward direction even after the concentration of auxin in the receiving block has become higher than that in the block supplying the auxin.

Suggestions concerning the mode of action of auxins on enlarging cells. Up to the present we have been considering the effects of auxins on cells in the regions of enlargement of growing organs. Anatomical investigation has revealed that in whole coleoptiles longer than about 12 mm., and such were used in all the early experiments, cell division had practically ceased. Since the meristem is apical,

cell division could play no part in the growth of a decapitated coleoptile of even shorter length.

F. W. Went suggested that auxin acts on cells undergoing turgor expansion in the oat coleoptile by rendering the cell walls more plastic. This would cause a decrease of wall pressure which, by enhancing suction pressure, might well lead to the initiation of turgor expansion. The effect of auxin on the extensibility of growing members has been investigated. Söding found that intact coleoptiles when acted on by small weights could without breaking be stretched or bent to a greater extent than decapitated coleoptiles; and Heyn has reported that decapitated coleoptiles covered with agar blocks could be stretched or bent to a greater extent when the blocks contained auxin (see Snow, *275*).

F. A. F. C. Went pointed out that auxin need not necessarily act directly on cell walls. It might, for example, affect the permeability of protoplasm, and, consequently, the rate at which an enzyme that can alter the plasticity of cellulose reaches the cell wall.

Other ideas are engaging attention (see van Overbeek, *222*). Some evidence has been obtained that auxins may serve as coenzymes for certain oxidation systems. Common and Thimann reported that the addition of auxin enhanced the oxygen uptake of coleoptile sections placed in solutions containing C_4 acids (*e.g.*, malic and fumaric acids). It is conceivable that auxin action is only manifested when the complete holoenzyme is formed. Apoenzyme may be present in the enlarging cells, and the arrival of the auxin coenzyme, migrating basipetally, may lead to the constitution of the operative holoenzyme.

It has also been asserted that the absorption of water by potato tissue is accelerated by adding auxin to the system. According to van Overbeek the osmotic pressure is not increased, and he considers that there may be a dual effect; wall pressure may be decreased, and that fraction of water absorption which is under metabolic control (see Bennet-Clark's experiments, p. 78) may be accelerated.

The problem under discussion in this subsection may well prove as difficult as all other problems of the behaviour of living cells in response to stimuli. In growth, the enlarging cells represent the regions of response, and auxin provides the chemical stimulus. A satisfactory explanation of the mode of action of auxin must account for the different responses of the enlarging cells of root and shoot, and for the influence exerted by light on the sensitivity of cells to auxin (p. 407).

Auxins and bud development. We have seen (pp. 347–8) that Snow obtained evidence that the influence inhibitory to the growth of the lateral buds of certain seedlings comes from the developing terminal bud, and is chemical in nature. Thimann and Skoog have more recently demonstrated that the continuous application to the top of a beheaded young shoot of *Vicia faba* of blocks of agar jelly containing an auxin occasioned strong inhibition of the growth of the lateral buds. In the control experiment blocks free from auxin were used, and the lateral buds grew into shoots. It thus seems that in growing plants the hormones that stimulate cell division and cell

enlargement (*e.g.*, in the developing terminal buds of seedlings) surprisingly possess the additional function of causing, either directly or indirectly (see Snow, *279*), the suppression of the growth of lateral buds, *i.e.*, of controlling that form of correlative behaviour known as apical dominance.

Auxins as phytohormones. We may regard auxins as phytohormones (p. 364). Auxins, in controlling growth in length, in regulating bud development, and, as we shall point out below, in stimulating adventitious rooting, etc., travel in polar fashion from a zone of supply to a zone where they exert a specific function. A notable point is that auxins, unlike food substances, produce their effects when acting in very low concentrations. For example, Kögl and Haagen-Smit calculated that 1 mg. of crystalline auxin-a possesses a strength of 30,000,000 A.E.

B. Auxins and Cell Division

It was at first thought that the sole function of auxin was to promote cell enlargement. This view has now been modified as we shall indicate in this and the next subsection. The evidence now to be presented suggests that auxins contribute in some way to the initiation of meristematic activity in a variety of plant organs. It is knowledge gained in the 1930's that has revealed the occasional dependence of meristematic tissue not only on the translocation of foods from green leaves and storage organs, which is the primary requirement, but also of auxins or of other special accessory substances (pp. 382–4). Such meristems as can synthesize auxins, or other spare parts of the protoplasmic machine, from foods will, of course, not be dependent upon migrating accessory substances, *i.e.*, upon hormones as defined on p. 280.

Callus formation, galls and root nodules. Laibach obtained the first evidence that meristematic activity may be stimulated by auxins when he discovered that cell division in pith and cortex, leading to general proliferation and callus formation, and sometimes also to the production of lateral roots, took place in young stems which had been smeared with lanolin containing auxin.

A simple class experiment is to induce callus formation in a young broad bean seedling by decapitating it, and smearing the cut end liberally with heteroauxin paste. The pot containing the bean is then placed under conditions favourable for growth. During the course of a week the smeared apex becomes swollen. If transverse sections of the apex are examined under the microscope it will be seen that irregular cell divisions have occurred, particularly in the cortex. In the control experiment, lanolin without heteroauxin is used, and, as a rule, no irregular cell divisions occur. It is usually observed that in the control experiment lateral buds, which remain dormant in whole plants, show much more growth than do those in the plants treated with heteroauxin paste. This experiment, therefore,

also serves to illustrate the phenomena discovered by Thimann and Skoog (p. 373).

Beyerinck (*29*) investigated the production of cecidomid galls in *Poa nemoralis*, and was fifty years ahead of his time in his interpretation of what he observed. He compared gall production to callus formation, and postulated as the exciting cause a substance coming from the larval body. He stressed the point that the galls can give rise to roots, of which some display normal structure and function, because he thought this meant that the substance of a living animal could in a few days so act upon a plant tissue as to evoke normal organ building. He regarded this action of animal substance as analogous to that of the inner causes which lead to root formation in uninfected normal plants. Later he developed the idea that these inner causes are special growth substances.

In recent years gall formation has been artificially induced by treating certain plant parts with heteroauxin. In his massive work on "Biochemistry and Morphogenesis," which is mainly concerned with the normal and morbid growth of animal tissues, but is rich in information and ideas of great interest to all biologists, Needham (*206*) gives examples of induced gall formation. Van Overbeek (*222*) refers to more recent work on this subject. He also cites evidence which conflicts with Thimann's hypothesis that indole acetic acid, passing out from Rhizobium bacteria, may form part of the reaction system which initiates the formation of root nodules in leguminous plants.

Cambial activity. In the 1890's Jost (see *160*) carried out excision and incision operations on epicotyls of runner bean seedlings in such a way as to obtain evidence that the production of vascular bundles by procambial and cambial cells in the epicotyl depends upon an influence, coming from the developing leaves, which migrates in a morphologically downward direction. Jost's views were not acceptable to some of the physiologists of that period. In an unpublished paper he cautiously stated that if a substance is discharged by the young leaves, it cannot be a nutritive substance in the ordinary sense of the term, but must be more akin to a specific substance such as Sachs had in mind (p. 349) or to a growth substance as defined by Beyerinck (see above). In the 1904 edition of his "Lectures on Plant Physiology," Jost compared the influence exerted by a growing leaf on cambial development with that exerted on plants by developing gall insects, and saw in their similarity a suggestion that a chemical substance acted as the stimulus which excited cambial divisions.

Jost remarks that a new interest was aroused in his old experiments with the discovery of the polar migration of auxins from developing leaves. The idea took shape that auxins might be the substances whose possible existence he had postulated forty years earlier. Jost gives to Snow the credit of providing the first piece of evidence in support of this idea.

Continuing his investigations on the nature of the cambial stimulus (p. 349), Snow (*278*) observed that a steady supply of auxin-a at 1/1,000,000 mg. per hour, or of heteroauxin at 1/500,000 mg. per hour, from a gelatin block, which had been affixed to the cut end of a split or decapitated shoot of a sunflower seedling, stimulated normal meristematic activity in the cambium.

After writing with immense authority about the development of our present knowledge of the stimulation and control of bundle building, Jost (*loc. cit.*) gives accounts of experiments carried out in the late 1930's at Heidelberg. He removed shoot tips and single leaves from the epicotyls of runner bean seedlings, and observed the resulting effects on growth and bundle building along the whole length of the epicotyl. He then determined how these effects were modified by the application of strong and weak heteroauxin pastes. He concluded that auxin promotes the building up of vascular bundles, but pointed out that the bundles produced in his experiments immediately below a decapitation wound did not look normal. Moreover he stated that growth in length did not proceed with the same cadence when decapitated parts were supplied with auxins as when buds were in position. He was inclined to support a suggestion made earlier by Söding that a second hormone may be necessary for normal morphogenesis in a bundle.

Jost also attached importance to the facts that bundle building increases towards the base of whole runner bean epicotyls and that this happens also in decapitated epicotyls. He cited evidence obtained by Söding and by Münsch that the cambium of trees contains and can manufacture auxins and, possibly, other hormones, and suggested that the cambium of runner bean is always producing hormones and discharging them downwards, and not only when it has been excited by receiving auxin from above. This suggestion seems to imply that the main function of the hormones that migrate from the developing apex of the epicotyl is to activate the differentiation of procambial strands and, possibly, of cambium in the leaf-trace area.

Clearly there remain for solution many difficult problems concerning this clear-cut example of morphogenesis in the plant body. Do specific chemical substances determine the retention of meristematic power in cambium, and the differentiation of newly formed daughter cells, not distinguishable in structure under the microscope, on the one side into phloem elements, which are themselves diversified, and on the other into the different elements of the xylem ? Are the daughter cells of cambium actually different, with their future fates determined, when cell division is consummated, or is their differentiation into permanent elements determined later by their position in the organism ? The matters discussed by Needham (*206*), and the amusing story in the footnote on p. 101 of his book, bear on this and on all examples of cell division and differentiation in the plant body.

Auxins and the formation of adventitious roots. In considering questions of polarity, particularly those exemplified in the rooting of cuttings, Sachs postulated the acropetal movement of substances specially fitted for the formation of buds and shoots, and the basipetal migration of those which determine root formation. In an elaborated form these ideas persist in Went's writings (for references, see van Overbeek, *222*). Went employs the general term *caline* to describe these substances, and distinguishes between rhizocaline, caulocaline, and other calines. Howard (*142*) and other workers consider that migrating auxin may be the sole determiner of types of meristem, and that " meristems develop into shoots when the auxin concentration is low and into roots where the concentration is relatively high."

In the present section we are concerned, not with the development of root initials already present in a cutting, but with the initiation of cell divisions in the pericycle. Van der Lek (see Went and Thimann, *328*) obtained evidence that an influence capable of promoting pericyclic divisions sometimes travels in extracambial tissues from buds, especially when they are sprouting. Having in mind Haberlandt's wound hormone, he postulated that the formation of adventitious roots is initiated by the reaction between pericyclic cells and a hormone which travels from buds, or developing shoots.

F. W. Went obtained the first unambiguous evidence of the root-forming action of a chemical preparation, when he stimulated root-formation in defoliated and decapitated twigs of Acalypha, by applying to the top of the stem agar jelly containing a water extract of leaves. Thimann and Went later found that certain products containing auxin can initiate and stimulate rooting, and Thimann and Koepfli have since demonstrated that crystalline auxins are also effective. The production of adventitious roots was observed by Laibach in his experiments with heteroauxin paste (p. 374), and by Snow in his investigations on the stimulation by auxins of cambial divisions (p. 376). By the middle 1930's physiologists, by their purely academic researches, had thus obtained good evidence that auxins may be one of the components of the complex reaction system that initiates pericyclic divisions.

Extensive trials have since been carried out in order to determine whether the promotion of rooting by traces of auxins, or of synthetic substitutes (see Nicol, *342*), has possible horticultural applications in the propagation of plants by means of cuttings. Some of the earlier trials were carried out in this country by Tincker and Unwin (*313*) and by Pearse (*227*), and in the U.S.A. by Hitchcock and Zimmerman, and by Cooper (see Thomas, *306*). During the war years many papers have been published in the *Botanical Gazette*, the *American Journal of Botany*, and other American periodicals. Some important successes have been achieved, but certain plants (*e.g.*, varieties of apple) have proved refractory. The system involved is complex and not yet under intellectual or practical control.

It is possible that traces of special substances (*e.g.*, thiamin, see p. 384) other than auxins may be essential. Went appears to consider that his postulated rhizocaline is the effective agent in promoting pericyclic divisions, and that natural or synthetic auxins act indirectly by mobilizing rhizocaline.

Auxins and the development of fruits. The existence of parthenocarpy in which seedless fruits are produced without fertilization (see Gustafson, *106*) must suffice us here as evidence of the view that the proliferation of ovarial and receptacular tissues is an after-effect of pollination, and may occur even if fertilization is not consummated. In the description of plant behaviour the term hormone was employed for the first time in 1909 by Fitting, who discovered that a chemical substance (which undoubtedly was an auxin), contained in the pollinia of certain tropical orchids, influenced the growth of the central column of the flowers of these orchids.

Gustafson gives accounts of experiments leading up to his own in which he induced parthenocarpy in a number of plants (*e.g.*, tomato) by applying auxin pastes to the tops of ovaries, after he had cut off the stigmas and styles. The development of this work (see van Overbeek, *222*) has led to the formulation of hypotheses about what may happen under natural conditions. Calculations indicate that the total amount of auxin in such pollen grains as may germinate upon a stigma is insufficient to stimulate the cell divisions and enlargements necessary for the formation of fleshy and other fruits. It has consequently been suggested that, in addition to containing free auxin, pollen may produce a substance which causes auxin precursor in ovarial and receptacular tissues to be converted into free auxin, which will then act as did the strong auxin in the pastes used by Gustafson in his experiments on induced parthenocarpy. According to this hypothesis fruit may form without fertilization, provided the pollinated flower remains on the parent plant and does not fall off. When fertilization occurs the maturing ovules produce further free auxin and possibly more of the substance causing the conversion of precursor to free auxin. Accordingly the setting of good fruit may be more frequent when fertilization and embryo development follow pollination.

Auxins and the inhibition of the formation of abscission layers. In summing up knowledge gained in the last century on the subject of abscission and leaf fall, Pfeffer (*228*, vol. II, p. 218) wrote " leaves fall and die . . . when they are prevented from exercising their normal functions. Similar factors, combined with correlative influences, determine the fall of unfertilized flowers, of fruits in which the ovules have been devoured by grubs, and of petioles deprived of their laminae." Evidence has now been gained that auxins play a part as factors or correlative influences in these phenomena (for biblio-

graphy see Gustafson, *106*, and van Overbeek, *222*). For example, the petioles of Coleus leaves, whose laminae have been removed, are retained on the plant for longer periods if the cut ends are smeared with heteroauxin paste. It has been inferred that in a whole leaf the basipetal migration of auxin from the blade retards the production of the abscisse layer and the subsequent changes in the cells which lead to abscission. Further, it has long been realized that withered flowers may drop when ovarial growth ceases because pollination or fertilization is not effected, and that, conversely, ovarial growth delays abscission. The delay of abscission is not dependent upon fertilization, for parthenocarpic fruits, produced naturally or by Gustafson's method of smearing ovaries with auxin paste, remain on plants until they are mature. The inference drawn is that free auxins migrate down the peduncle from the developing fruits and in some unknown way prevent abscission.

It has been reported that in the U.S.A. there is under trial a practice of spraying with synthetic auxin certain varieties of apple which are notorious droppers, and that the dollar return of this practice has already been substantial. And, branching from the academic study of the transmission of the phototropic stimulus (p. 365), there are other sidelines of investigation that may have economic importance, besides those on the spraying of fruit trees and on the rooting of cuttings. For example, much publicity was given in the spring of 1945 to the possible practice of treating cornfields with special chemicals possessing some of the properties of auxins (*e.g.*, the methoxone of the Imperial Chemical Industries Ltd.), which, it was asserted, kill certain dicotyledonous weeds without injuring the growing corn. Reports of a co-operative scientific research that bears on this important practical possibility will be found in *Nature*, vol. 55, p. 497.

C. Bios and the Growth of Yeasts

Pasteur's researches appeared to give conclusive evidence that all races of yeast can grow in culture solutions containing nothing but a sugar and certain mineral salts; but, early in the present century, Wildiers reported that in his experiments minimum amounts of inocula had to be exceeded for successful growth. He suggested that some of the organisms died, and that their remains provided a special substance, which was essential for the growth of those which survived. He called this substance *bios*. He was virtually suggesting that, in addition to the recognized food substances, yeasts may require accessory substances of the kind now called vitamins by students of animal nutrition. After many years of controversy concerning bios, it appears now to be settled that some of the wild yeasts can grow on media akin to Pasteur's solution in the absence of bios factors, but that some of the cultivated yeasts must be provided from outside with accessory substances. The general idea is that all yeasts require these substances, but the wild yeasts can synthesize them all

from sugars and salts, whereas the cultivated yeasts have synthetic disabilities. Different races of yeasts may have different disabilities.

It has been revealed by growth experiments and chemical analysis that the bios of Wildiers is a complex of several substances. What was at one time called bios I is now known to be inositol, and bios II is the substance called biotin. Among the other substances constituting a whole metabolizing intracellular system requisite for growth, which a cultivated yeast may fail to synthesize for itself, are nicotinic acid, riboflavin (vitamin B_2), pantothenic acid, and pyridoxin (vitamin B_6). Such failure means that for growth the wanted substance must be present in the environment.

The discovery and isolation of biotin by Kögl and Tönnis was a fine bit of biochemical work, demanding high chemical skill in extracting from massive amounts of material a pure product which was present only in vanishingly small quantities. A certain race of cultivated yeast, which lacked bios factors, was used as the test object. A culture solution of pure sugar and minerals was inoculated with a given amount of yeast suspension, and the mixture was shaken for a period at a constant temperature. The amount of growth was judged by measuring with an extinctiometer the increasing cloudiness of the solution. A Saccharomyces unit (S.E.) was defined as that quantity of added accessory substance which brought about a hundred per cent. increase in cloudiness. In preliminary experiments it was ascertained that egg yolks have the very high activity of 300,000 S.E. per gm. Later, 1·1 mgs. of a crystalline compound were obtained from 250 kgs. of chinese egg yolks. This compound was the methyl ester of biotin, and the formula suggested for biotin was $C_{10}H_{16}O_3N_2S$. It has since been shown (see Openshaw, *217*) that biotin is identical with vitamin H, and a structural formula has been assigned to it (p. 445). Kögl reported that in concentrations as fantastically low as 1/400,000,000,000 his crystalline biotin occasioned detectable accelerations in the growth rate of yeast.

D. Demands on the Environment made by Certain Fungi and by Bacteria for Accessory Substances

If we regard protoplasm as a chemical machine in which a large number of enzymic, coenzymic, and other spare parts must be assembled before it will assimilate the substances traditionally looked upon as its foods, and so build up more protoplasm and other cell components, we may conclude that in growth either protoplasm must have the power of making all its own spare parts from the absorbed foods, or else its environment will have to supply ready made such spare parts as it cannot synthesize. For example green plants can grow when there are no organic substances in the environment, but animals require vitamins in addition to proteins, carbohydrates, fats, mineral salts and water. Some at least of these vitamins may be regarded as spare parts of growing or functioning protoplasms. It is suggestive that some of these vitamins (*e.g.*, aneurin (vitamin B_1), and riboflavin (vitamin B_2)) have affinities to components of enzymic

systems whose metabolic functions in oxidation, decarboxylation, etc., are under investigation.

Evidence has been obtained that certain fungi, *e.g.*, *Aspergillus niger*, can grow on media containing only sugar and mineral salts, whereas others require the presence in the environment of traces of additional substances. For example, *Phycomyces blakesleeanus* makes little growth if it is not supplied with aneurin. (Thiamin is yet another name for this substance.) Under certain standardized conditions the rate of growth of this mould may be controlled by the amount of thiamin supplied to it. Indeed, assays of the vitamin B_1 content of animal foods have been made by measuring the stimulating effect on the growth of this mould caused by adding extracts of the foods. The results are expressed as Phycomyces units. The general conclusion drawn is that *Aspergillus niger* can synthesize all its spare parts from food-materials, and that *Phycomyces blakesleeanus* has the single synthetic disability of failing to manufacture the necessary substance thiamin, which must be supplied therefore by the environment. Robbins and Kavanagh (*247*) have written a review about the synthetic disabilities of some fungi.

Stephenson (*283*), in summarizing knowledge of the growth factors for bacteria, has stated that most of the vitamins known to function in the animal have been found necessary for one bacterial organism or another. She points out that some bacteria require substances which have not yet been proved to be necessary for any animal, but recalls that certain substances (*e.g.*, biotin) were shown to be bios factors for yeast before they were identified as vitamins for animals. The idea of "one kind of active matter" referred to on p. 2 of this book is finding a wider application than the natural philosophers of the nineteenth century had in mind.

Miss Stephenson mentions provocative suggestions made by Fildes, namely that primitive organisms were completely self-supporting (*i.e.*, they displayed autarchy), and that along certain lines of evolution synthetic powers have been lost. The argument goes on with the corollary, the greater is this loss the greater will be the number of compounds required from the environment in order to build up cell materials. Loss of synthetic powers is attributed to the growth for prolonged periods of certain organisms in environments containing a rich assortment of molecules which they are able to take in "ready made". The Lamarckian flavour of the idea of loss by disuse of synthetic powers should be noticed.

E. The Autarchy of Green Plants

When growing in the complete absence of organic substances in the environment, green plants display their power of synthesizing not only the traditional food materials but also most of those special substances which, in the study of animal nutrition, have been classed as vitamins (see Rosenberg, *252*). They manifest their complete independence of ready made spare parts, *i.e.*, their autarchy. We are still ignorant of the function exercised in green plants by most of these spare parts. Some of them (*e.g.*, thiamin, ascorbic acid, riboflavin) are components of enzymic systems.

To state that a whole green plant can synthesize a certain vitamin (*e.g.*, thiamin) leads to the question, is the power to synthesize it possessed by

all the living cells, or is there a division of synthetic labour among the tissues ? More particularly we may ask—what are the synthetic disabilities of fertilized egg cells, and each of the various meristems produced in succession from them during the development of the embryo, the germination of the seed, and subsequently ? And, if such disabilities are detected, from what anabolizing tissue (*e.g.*, mesophyll, storage tissue) do the essential ready-made spare parts migrate ? Clearly, questions posed in the discussion on auxins (p. 376) may also be put for some of the other growth substances. Such of these as are proved to migrate in the organism may be permissibly described as phytohormones. It should be noted, however, that such migratory substances would be derived ready made by the meristematic tissues from their internal environment. Accordingly these tissues would be related to the special substances in much the same way as cultivated yeasts are to bios factors. It is only in the relation of the whole organism to its external environment that the distinction between the terms, growth hormone and bios or vitamin, has much significance.

The alternatives are obvious. A meristematic cell may resemble some of the wild yeasts and *Aspergillus niger* in being able to manufacture all its own spare parts from the recognized food materials, or it may resemble animals, bacteria, some of the cultivated yeasts, and *Phycomyces blakesleeanus*, and require a supply of one or more spare parts, ready made. So far, apart from what has already been written about auxins, we can only cite evidence obtained from experiments on the growth of embryos and of root tips in culture media.

F. The Growth of Excised Embryos in Culture Media

In the last century van Tieghem reported that excised embryos showed growth when placed in pastes made from cotyledons or endosperm, and Brown and Morris proved later that growth might occur on aqueous media containing carbohydrate and mineral salts. Similar experiments were carried out in the 1930's with purer chemicals in media kept freer from bacterial infection (for bibliography, see Bonner and Axtman, *39*). Ray reported that excised pea embryos grew on a gelatin gel containing cane sugar and essential mineral salts, without the addition of accessory substances, but Kögl and Haagen-Smit found that growth was promoted by the addition of traces of biotin and thiamin. According to Bonner and Axtman pea races may vary in that the embryos of only some of them respond with enhanced growth to the addition of accessory substances. Where growth has been enhanced, a tentative conclusion is that biotin directly stimulated shoot growth, and thiamin primarily promoted root growth (see below). Such promotion would augment the absorption of sugar and salts from the medium, and, in consequence, the shoot would then grow faster.

By measuring S.E. values (see p. 380) of seed extracts, Kögl and Haagen-Smit obtained evidence of the presence of biotin in all the seeds they examined. It has long been known that seeds contain thiamin. Accordingly we may with reason suppose that during the germination of seeds not only the recognized food substances are

mobilized but also certain stored accessory growth factors, and that these factors migrate to the meristems of plumule and radicle as ready-made spare parts, or as their close precursors (*cf.* remarks about auxin precursors on p. 371). It is difficult to prove that these meristems are completely dependent upon such migration, since excised embryos usually contain at the outset of experiments accessory substances that accumulated during their development before the seeds were shed. In order to prove conclusively that a substance is an essential factor it must be shown by experiment, not only that the substance stimulates growth, but that no growth occurs in its absence. We have no such proof that biotin is an essential growth factor in the higher plants, but, as we shall now see, there is good evidence that the growth of at least some roots is dependent upon a supply of thiamin.

G. The Growth of Roots in Culture Media

In the years 1922–23 Robbins reported the results of experiments in which he caused excised root tips of tomato to survive and grow for a period of five months when kept under certain special conditions. The problems raised by this important discovery have been discussed and further investigated by White (*332*). He placed excised root tips of tomato in about 50 c.c. of a balanced nutrient solution containing the essential inorganic ions, two per cent. sucrose, and 0·01 per cent. of *dried brewer's yeast.* He passed the roots into fresh solutions every week. The root tips grew actively for over a year. It was calculated that a single initial fragment 10 mm. long, produced 35,000 growing points and a branched root system 400,000 mm. long. Important inferences concerning the metabolic powers of root tips have already been made (p. 205) from the fact that the entire material of the branched root system must have been derived from the nutrient solution, the composition of which was known excepting for possible impurities in the minerals and for the components of the dried yeast. Very little growth occurred in the absence of dried yeast; hence great interest attaches to the identity of these components of yeast, which, in low concentrations, promote the growth for unlimited periods *in vitro* of root tips of tomato and of other plants, when these root tips are supplied with the necessary materials for tissue construction. The possibility of replacing yeast extract by "more easily analysable material" was considered by White, and reports of more recent work in this direction suggest that important successes have been achieved by Robbins (see *248*), Bonner, and others.

The evidence strongly suggests that *thiamin* (*i.e.*, aneurin or vitamin B_1, see p. 445) acts in plants as a specific hormone in promoting the growth of some roots. Bonner (*37*) found that pea root tips grew for three weeks in a solution containing essential inorganic ions

and sucrose, but the growth rate gradually declined to zero. The thiamin content of the roots, as estimated by a biological method, was found to decline simultaneously. Furthermore, using comparable samples, he showed that during the second week whereas the growth rate was only 10 mm. per week in the thiamin-free solutions, it was 60–100 mm. per week in solutions containing thiamin. Molar concentrations of 10^{-7} or less were found to be effective. The root appears to require the whole vitamin B_1 molecule, since neither the pyrimidine nor the thiazole components, when supplied alone, promoted growth. Inasmuch as stimulations occurred when both components were added to the nutrient medium, it was inferred that roots can synthesize vitamin B_1 from its components. Another inference is that pea roots cannot synthesize from sugar and mineral salts either of the components of vitamin B_1. The conclusion bearing on the subject matter of this section is that some roots are dependent for their growth on a trace of thiamin (or of each of its components), which migrates downwards from the shoots, *i.e.*, which acts as a correlator.

It has been found that the rate of growth of roots *in vitro* is less when stimulation is brought about by thiamin than when dried yeast is used, clearly suggesting the existence of other growth factors. These do not appear to be amino acids (which, as White has shown, may stimulate root growth) or trace elements. Bonner (*38*) concluded from his experiments that isolated roots placed in culture solutions do not suffer from biotin deficiency. He obtained evidence that the roots synthesized this growth substance. There is some evidence (see, *e.g.*, Bonner and Devirian, *40*, and Bonner, *38*) that nicotinic acid may in certain plants migrate as an additional hormone from the shoot system and stimulate the growth of of some roots. Robbins and Schmidt (*249*) reported that vitamin B_6 (adermin) is a growth substance for some tomato roots. Bonner (*38*) confirmed this finding, and stated that this substance also stimulated the growth of roots of carrot and *Datura stramonium*, but not that of roots of clover or cotton. It appears, therefore, that, as in different species and races of fungi and bacteria, so also in root meristems of different species and races of flowering plants, there may exist qualitative as well as quantitative differences in synthetic powers and disabilities. Indeed there were indications in one of Bonner's experiments that some roots can synthesize thiamin and, in consequence, grow in culture media which do not contain this growth substance.

H. Other Growth Substances

Haberlandt's wound hormone (see p. 349). Substances possessing the properties of wound hormones have been called *traumatins*. English *et al.* (*82*) have isolated from plant tissue a dicarboxylic acid, $HOOC.CH = CH.(CH_2)_8.COOH$, which evoked the formation of phellogen and wound periderm in washed discs of potato tissue. They synthesized this acid, and the synthetic acid had properties identical with those of the natural product.

Hypotheses concerning hormones and the differentiation and growth of leaves and reproductive organs. We have no room for more than a few short statements about this highly important and interesting subject. We shall

first state an established fact. Purvis and Gregory (*241*) found that the developing shoot axis of winter rye produces first of all about seven obligate leaf primordia and then about eighteen labile or facultative lateral primordia, whose differentiation is controlled by the temperature and the photoperiod. These workers state that the labile primordia possess a double structure, which enables them alternatively to develop into leaves or into spikelets. Subsequently only obligate spikelet primordia are formed.

It has long been recognized that internal causes, conditioned by environmental influences, determine whether such lateral primordia as are produced by a meristematic shoot apex will develop into leaves or reproductive organs, but it is a modern hypothesis that such differences in morphogenesis may be controlled by the action of hormones. This hypothesis has been used to interpret some of the results obtained in experiments on vernalization (for a review of work on this phenomenon and a well-selected bibliography, see McKinney, *198*) and on photoperiodism (see Hamner, *113*). For example, from the results of their experiments on the vernalization of excised embryos placed in culture solutions, Gregory and Purvis (*104*) have inferred that the growing embryo of winter rye can synthesize a hormone precursor (A), whose biochemical fate will depend upon external conditions. They postulated that at low temperatures, during vernalization, A may be converted into a substance B, whose subsequent fate will be governed by the photoperiod and the temperatures prevailing during the light and dark periods. It may be converted into a spikelet-initiating substance C and a spikelet-maturation substance D, or into a substance E which promotes leaf formation. Other suggestions concerning the possible existence of hormones which promote leaf growth are referred to by Bonner and Haagen-Smit (*36*), and of hormones which promote flowering by Went (*327*). Touching *florigen*, the flower producing hormone, which, so it has been alleged, may be synthesized by leaves during a photoperiod, we quote from p. 585 of Hamner's review : " Proof of the existence of a hormone has not yet been forthcoming, since no one has successfully extracted a substance from photoperiodically-induced leaves, identified and synthesized it, and reapplied it to vegetative plants, thereby inducing floral formation. In fact, so far as is known, no one has obtained an active extract from flowering plants which will result in floral initiation in vegetative parts." Hamner virtually states the exacting tasks which confront future workers in this important field of investigation.

CHAPTER XVIII

PLANT MOVEMENTS [1]

A. Notes Leading to a Classification of Plant Movements

IT has long been recognized that plants possess irritability and execute movements in response to a variety of stimuli. "An organism moves according to its inherent nature and the means at its disposal," *i.e.*, specific irritability and general structure both contribute in determining the kinds of movement a plant may display.

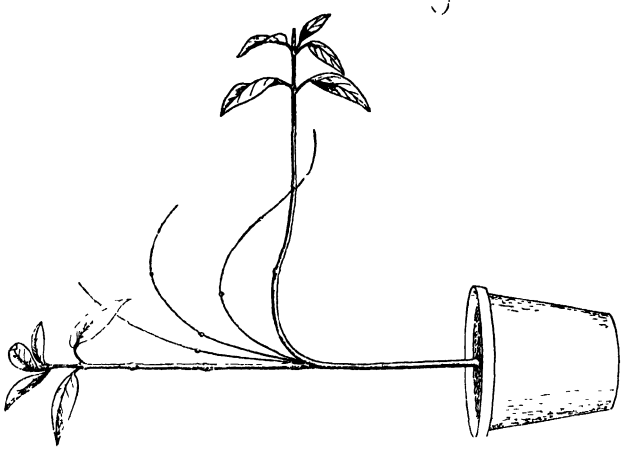


FIG. 60. Negatively geotropic curvature of a stem brought about by unequal growth rates on opposite sides of such internodes as are capable of growth. Over-curvature is shown before the vertical position is assumed.

Thus, owing to the limitations imposed by structure, the only possible movements from place to place in a rooted plant are those which occur inside living cells. It must be remembered, however, that locomotions are shown by ciliated plants, gametes, and zoospores, and that the plasmodia of Myxomycetes display creeping movements.

[1] We shall consider only such movements as are induced when stimuli act on irritable protoplasm (p. 4), and shall not discuss the purely mechanical movements that are associated with the dehiscence of sporangia and fruits, the dissemination of spores and seeds, and so forth.

For a full account of the earlier work on plant movements see Pfeffer (*228*, vol. III) and Jost (*158*), and for considerable treatment of old and newer work see Stiles (*291*, pp. 431–544): and for the experimental study of this subject see Darwin and Acton (*71*) and Small (*271*). The account given in the present chapter of recent advances in our knowledge has been based on Went's presentation of the subject (see Kostytschew, *174*); the monographs of Boysen-Jensen (*45*) and Went and Thimann (*328*) should be consulted for fuller information.

When opposite sides of a fixed member of a plant grow at different rates, a movement known as a growth curvature is executed (fig. 60). Fully grown portions of plant members do not as a rule possess means for showing active movement; but in a limited number of plants belonging to several families (*e.g.*, Leguminosæ) specialized motor organs called pulvini occur as swellings at the bases of leaves and petioles. In *Mimosa pudica* the walls of the parenchymatous cells are thinner in the lower than in the upper half of the pulvini. The intercellular spaces are large and there is a central vascular bundle in each pulvinus. Bending movements of whole leaves, leaflets, and pinnules, are brought about by changes of turgor on the two sides

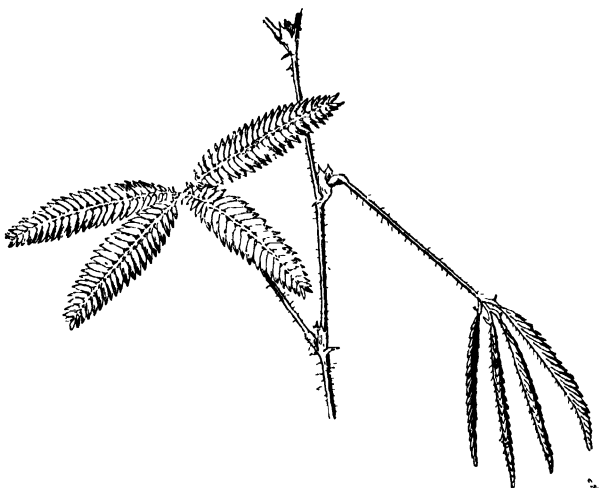


FIG. 61. Movement of variation shown by *Mimosa pudica.*

of the pulvini. When Mimosa is stimulated water escapes into the intercellular spaces and there occurs differential loss of turgor in the cells of the lower half of the pulvinus. Tissue tensions exist in pulvini before stimulation, as may be readily shown by the usual method (p. 471); so it is not surprising that, after stimulation, the interplay between the tensions in the upper half of the pulvinus and the elastic recoil of the cell walls in the lower half leads to a change in the shape of the pulvinus, and to movement. Such a pulvinar movement is termed a movement of variation (fig. 61).

A movement induced by a stimulus acting from within a plant is described as spontaneous or autonomic, and external stimuli are said to evoke paratonic movements. Many locomotions are autonomic, and the periodic or nutational movements shown by the growing shoot apices of plants are autonomic growth movements. The stimulus comes from within, and response takes the form of a differen-

tial growth rate, resulting in the progression of the more rapidly growing zone around the apical region. Spontaneous movements of variation appear to be shown by all plants possessing functional pulvini, but they are not easily recognized when paratonic movements of variation are superimposed on them.

The orientation of plant members is, however, largely determined by external stimuli. When an equilibrium condition is disturbed either by an alteration in the position of a plant member or by a change in environmental conditions, a paratonic movement takes place until equilibrium is once more established. When the new position assumed by a member shows a relation to the direction of application of the stimulus, we speak of the movement as a tropism. For a tropic response to occur, the external stimulus must be essentially unilateral. The stimulus must either exist on one side only of the irritable member, or be in greater effective quantity on one side than on the other. According to older views a certain minimum threshold difference must be exceeded before an irritable plant member can discriminate between differences in intensity of a stimulus that impinges upon opposite sides of the member. It was agreed that a difference in the intensity of the stimulus beyond this threshold value caused a greater physiological disturbance, and more marked after-effects; and much work was performed in attempting to show for plants that the excitation is proportional to the logarithm of the effective stimulus (Weber's law). Modern work (see *e.g.*, pp. 403–4) suggests that there are more productive ways of investigating the quantitative relation between a stimulus and the amplitude of the corresponding movement.

The other paratonic movements of fixed plant members are described as nasties. Nastic movements occur (*a*) when the stimulus is diffuse, *i.e.*, when it does not impinge upon the member from a definite direction, and (*b*) when the movement induced by the stimulus, whether this is diffuse or unilateral, is exclusively determined by the properties of the irritable member. Pulvinar movements are always nastic in nature, and nastic movements may also be brought about by growth.

We shall give examples below of tropic and nastic movements. It will be noticed that freedom to show tropic responses is possessed only by such members as can execute growth curvatures. When a plant organ grows more slowly on the side to which the tropic stimulus is applied, a positive tropic curvature results; when more rapidly, a negative tropic curvature occurs. Certain terms are useful in describing the position taken up by the organ when in equilibrium with tropic stimuli. Organs which, at equilibrium, lie in the line of action of the stimulus are described as orthotropic; and those which place themselves at an angle to this line are said to be plagiotropic.

Pulvinar movements are nastic since they are exclusively determined by the properties of the pulvini. Moreover, nastic responses by growth curvatures both to diffuse and to localized stimuli frequently occur.

The classification of plant movements given below has been constructed on the basis of what has been written in this section. The terms used in describing individual movements will be discussed in the next section, and some consideration will be given there to the advantages accruing to plants from certain of the movements.

CLASSIFICATION OF PLANT MOVEMENTS

A. Movements shown by freely motile organisms and free parts of fixed plants

(1) Autonomic locomotions (movements induced by internal stimuli). Examples: autonomic movements of motile algæ, fungi, spores, and gametes; the circulation of protoplasm inside living cells of higher plants; the movements of chromosomes during nuclear division.

(2) Tactic movements (taxis) (movements induced by external stimuli). Examples: phototaxis of motile algæ or of chloroplasts inside living cells of higher plants; chemotaxis of gametes.

B. Movements shown by fixed members of fixed plants

(1) Autonomic movements (movements induced by internal stimuli).

(*a*) Growth movements. Examples: growth itself; nutations; nastic bendings.

(*b*) Movements of variation. Examples: pulvinar movements of the terminal leaflet of red clover, and of the lateral leaflets of the telegraph plant (*Desmodium gyrans*).

(2) Paratonic movements (movements induced by external stimuli).

(*a*) Growth movements.

(i.) Tropic movements. Examples: geotropic, phototropic, hydrotropic, chemotropic, and haptotropic movements of members of shoot and root systems.

(ii.) Nastic movements. Examples: photonastic, thermonastic, and haptonastic movements of fixed members.

(*b*) Movements of variation. Examples: photonastic, haptonastic, and seismonastic, pulvinar movements of *Mimosa pudica* and other "sensitive plants".

B. Notes on Individual Tropic Movements

External unilateral stimuli evoke tropic responses or tropisms. Certain descriptive technical terms are in general use which indicate succinctly that there is a causal connection between an external condition and a directional response. Thus, the terms geotropism, phototropism (or heliotropism), hydrotropism, chemotropism, and haptotropism (or thigmotropism), describe movements induced by the external stimuli, gravity, light, water, chemicals, and contact, respectively. The use of such terms defines many problems, but the

movements so described cannot be explained until it is known how plants can receive and perceive external stimuli, and how, upon reception, chains of events are initiated, and subsequently governed until the tropism is displayed.

Geotropism. The stimulus of gravity must be unilateral since this force is exerted from one direction only. Primary roots are positively geotropic, secondary laterals are apogeotropic (plagiotropic to gravity) and directed downwards, and laterals of higher orders are ageotropic (insensitive to gravity). The result of this varied behaviour is advantageous to the plant, since a greater volume of soil, and therefore of water containing nutrient mineral salts, is tapped by the plant than if all the different orders of roots responded in the same manner. Spread of the shoot system is similarly effected when the main stem is negatively geotropic, the lateral branches apogeotropic and directed upwards, and the leaves transversely geotropic (*i.e.*, they are plagiotropic to gravity and place themselves at right-angles to its line of action). By this outward spread of members, leaves become placed in a better position for receiving light for photosynthesis than if all members reacted similarly to gravity.

There are many exceptions to the general mode of behaviour described above. For example, rhizomes and runners are plagiotropic to gravity, and the leaves of many plants, particularly among the monocotyledons, are nearly orthotropic.

The response of a given member to gravity may change during development, and biological advantages may accrue. For instance, poppy buds are positively geotropic and poppy fruits negatively geotropic. The young stems of twiners are negatively geotropic and show autonomous nutations. Later, the internodes towards the stem apex become plagiotropic to gravity, and when a position at a considerable angle to the vertical has been reached a new form of geotropic response is manifested. This is termed lateral geotropism. One side of each inclined upper internode grows more rapidly than the other, and a circular movement, by which supports are entwined, is thereby executed ; this may be clockwise (hop), or counter-clockwise (Convolvulus or Phaseolus). Since the autonomous nutation persists, torsional twists, which would otherwise occur in the stem as a result of the circular movement, are prevented. The advantage of the climbing habit is that assimilating systems are thereby borne above the general level of herbaceous forms, without the expenditure of matter and energy, in the production of mechanical tissue, that would otherwise be necessary to maintain a rigid erect posture in a tall shoot.

So-called readjustments of correlation may be observed when injury removes a leader bud of the spruce, or the tip of any primary root. In the one, the shoot springing from a lateral bud becomes negatively geotropic, and, in the other, two or more lateral roots may become positively geotropic. These purely automatic readjustments are obviously of great advantage to the injured plant.

The experimental study of geotropism began in 1809 when Knight fastened plants in various positions on a rapidly rotating wheel, thus submitting them to the action of centrifugal force. The stimulus was

the force which was equal in magnitude but opposite in direction to the centripetal force due to the rotation of the wheel. On vertical wheels the stems all turned towards the centre, and the roots away from the centre. Stems and roots, therefore, behaved towards centrifugal force as towards gravity. Organs fixed vertically on the rim of a horizontal wheel assumed intermediate positions, the final angle to the plane of rotation depending on the velocity of rotation. Plainly, the final position was a result of the combined effect of centrifugal force and gravity. Evidence was in this indirect way obtained that the gravitational force governs the orientation of plant members.

It was at one time thought that the downward curvatures of horizontally placed roots resulted solely from their own weight. But it is now well established that geotropic curvatures are possible only through growth. Roots in executing geotropic curvatures penetrate mercury, a liquid on which dead roots float. Furthermore, a crude mechanical explanation fails to account for negatively geotropic and diageotropic responses. Experiments with marked roots (fig. 62, A) or with stems (fig. 60) show (*a*) that only those regions capable of growth can execute geotropic curvatures and (*b*) that geotropic curvatures are brought about by different growth rates on the two sides of the irritable member. It clearly follows that when conditions are unfavourable for growth, owing, say, to absence of oxygen or to too low a temperature, geotropism cannot be displayed.

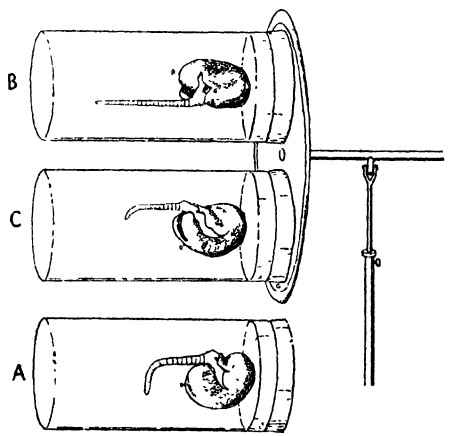


FIG. 62. (A) Positively geotropic curvature, of a root brought about by unequal growth rates in such zones as are capable of growth. (B) Neutralization of the effect of gravity by slow rotation on a horizontal klinostat. (C) Curvature shown by a root on a klinostat after previous stimulation for a period longer than the presentation-time.

The nodes of grasses provide an instructive special case. When a grass haulm is laid horizontally, gravity stimulates the lower sides of these nodes to grow anew. As the upper sides remain unchanged, a knee-like curvature is thereby produced, and the haulm again becomes vertical.

If general observations suggest that gravity governs a movement executed by a plant member placed in a horizontal position, confirmation should be sought by experiments with a horizontal klinostat (fig. 62, B). This instrument, of which there are many patterns,

consists essentially of a surface which is clock-driven at a slow rate about a horizontal axis. Plants are so affixed to the klinostat that the member under experimentation (*e.g.*, the main stem of a potted plant, or the radicle of a seedling) lies horizontally. Growth will continue, but geotropic curvatures will not occur, since, owing to rotation, gravitation acts, over a period, equally on opposite sides of the member. The unilateral effect of gravity, which would be exerted were the klinostat at rest, is thus annulled by rotation.

Phototropism. For light to act as a unilateral stimulus, the plant should receive light from one side only, or, the intensity of the incident light should be greater on one side of a plant member than on the other, as often happens, for example, in hedgerows and at the margins of woods. General observations in the field suggest that most stems are positively phototropic, and that it is usual for leaves to be plagiotropic towards light. The advantage gained by these responses is that assimilating members are brought to positions where light is plentifully received. Study of the leaf mosaic of sycamore shows that surprisingly little overlapping of leaves occurs. The final position assumed by a leaf depends on geotropic as well as phototropic responses. When the stimuli of light and gravity are antagonistic, as happens when vertical stems receive unilateral illumination, the light stimulus usually proves to be the more powerful of the two. Consequently, in executing a phototropic response a member may be removed from its equilibrium position towards the gravitational force.

Although the negative phototropism of the adventitious roots of root climbers (*e.g.*, the ivy) is an important property, little or no functional significance can be attached to the light responses of most roots, since they normally grow in the soil. Actually, most roots are insensitive. Negative phototropism towards continuous unilateral illumination of moderate intensity has, however, been observed in certain roots, (*e.g.*, those of sunflower, garden cress, white mustard).

Interesting changes in phototropic responses may occur during development. Thus the flowers of *Linaria cymbalaria*, a plant which scrambles on rocks, are positively phototropic, and this favours insect visitation. After fertilization the response to light becomes negative, so rendering it more probable that the seeds will be deposited in rock crannies, where this toadflax meets with less competition than in formed soil.

The continuous or intermittent illumination of a plant member [1] from one side only is easily arranged : consequently both the nature of the phototropic responses of members and the fact that all phototropic curvatures are growth curvatures may be readily demonstrated. The course of phototropic curvature is illustrated in fig. 54. It is

[1] Or of a vessel containing motile organisms (*e.g.*, Chlamydomonas) that possess phototactic irritability.

important for some purposes to realize that plants which are rotated on a vertical klinostat do not execute phototropic curvatures when the klinostat is subjected to unilateral illumination. Clearly, it is again a question of annulling a unilateral stimulus by rotation (*cf.* p. 391).

Hydrotropism. Soil may be wetter in certain localities than in others, and roots tend to grow towards the wetter regions. The positive hydrotropism of roots may be demonstrated by growing plants in moist fibre in a shallow sieve inclined to the vertical. The roots grow vertically downwards until they penetrate the sieve. They then by growth curvature bend back from the dry air towards the moist fibre, *i.e.*, hydrotropism proves more powerful than geotropism.

Chemotropism. Chemical substances in the environment have a directive influence on the growth of certain plant structures. Thus when pollen grains germinate on nutrient jelly in which pieces of pistil have been sown, the pollen tubes grow away from the air and towards the pieces of pistil. The tubes may be described as showing negative aerotropism and positive chemotropism. It is presumed that chemical stimuli are present in the pistils, and that, after pollination, these stimuli play an important part in directing the growth of the pollen tube.[1]

Haptotropism. Non-twining climbers gain the same advantages as twiners (p. 390). We are not concerned here with the plants that climb by means of aerial roots or hooked prickles, but with climbers which can make use of supports because they possess ordinary members (*e.g.*, the petiole of most species of Clematis, the leaf tip of Gloriosa, the root of Vanilla) or specialized tendrils (*e.g.*, the modified leaves of the garden pea, stipules of Smilax, and the branch of the vine) which are sensitive to contact with the uneven surface of solid bodies, and execute haptotropic growth curvatures. It is a remarkable fact that they are sensitive to contact with the surface of a glass rod, but not to that of a glass rod coated with gelatin gel (*i.e.*, a liquid system). Rain-drops do not stimulate them. It appears that haptotropism is displayed as a result of the shortening of the cells on the stimulated side of the sensitive structure, while the growth of the opposite side continues. A sharp curvature is thereby produced and the tendril, leaf tip, or petiole, coils round the object with which it has made contact. Tendrils continue to coil until they are fully grown, and the unattached part between the entwined object and the plant shortens into a double spiral, so bringing the plant closer to its

[1] A parallel phenomenon, viz., the chemotaxis of motile cells (*e.g.*, the spermatozoids of ferns), may be demonstrated by placing a fine capillary tube containing a solution of 0·01 per cent. sodium malate in a drop of water containing the sperms. The movement of the spermatozoids towards the mouth of the tube may be observed under the microscope.

support. If the climber sways in the wind, the spirally coiled tendril alternately loosens and tightens.

C. Notes on Individual Nastic Movements

Most of the facts mentioned in this section can readily be verified by observations in the field and in botanic gardens, or by simple experiments in the laboratory. Very diverse forms of biological advantage may accrue from nastic movements. Thus the movements shown by insectivorous plants lead to the absorption of nitrogenous foods. When the nastic movement of a leaf causes a reduction of the amount of leaf surface exposed, transpiration is diminished, and this may be a definite advantage when water deficits have arisen. Sometimes the movements of leaves may lessen the chance of injury to chloroplasts by over-insolation. Doubtless the movements of floral members increase the probability of fertilization, and are thus concerned with the survival of the race.

Nyctinastic movements. During a period of twenty-four hours changes of temperature and light intensity may induce visible nyctinastic movements. Thus by nyctinastic growth curvatures certain flowers can perform opening and closing movements; and nyctinastic movements of variation resulting from turgor changes in pulvini are shown by the leaves or leaflets of many plants in the Leguminosae (*e.g.*, clovers, acacia, and species of Mimosa), *Oxalis acetosella*, the telegraph plant (*Desmodium gyrans*), species of the fern Marsilia, etc. The individual factors, viz., light intensity and temperature, acting singly, may induce photonastic and thermonastic movements respectively. Thus before their petals are fully expanded the flowers of tulip or crocus will, at constant temperature, open when illuminated and close when darkened, and so display photonasticism, and will at constant light intensity show thermonasticism by opening in warm air and closing in cold air. Such photonastic and thermonastic movements are brought about by growth curvatures. When the lower side grows more rapidly, hyponastic curvature and closure follow, and epinastic curvature and opening result from more rapid growth of the upper side.

Haptonastic movements. When a marginal tentacle of a leaf of any species of sundew is stimulated by contact (*e.g.*, by a fly's body), a haptonastic growth curvature is executed, and the fly is thereby brought in contact with the small central tentacles. These thus become stimulated, and, as a result, other marginal tentacles execute growth curvatures towards the body of the fly. These later curvatures are more properly described as haptotropic since the inducing stimulus has a directional influence. The first movement of a stimulated marginal tentacle towards the central tentacles is, however, haptonastic, since it is exclusively determined by the properties of

the tentacle ; the stimulus exerts no directive influence. Stimulation by means of chemical substances evokes a much more vigorous response. Poisons such as mercuric chloride as well as nutrient substances may induce movement. Clearly it is not the advantage gained that causes such a chemonastic movement.

Mimosa pudica is the best known sensitive plant. It executes paratonic nastic movements in response to the stimuli of contact, shock, or injury. Remarkable pulvinar movements of variation can be induced by singeing a single terminal leaflet. The whole plant may be affected. In the stimulated leaves, the secondary petioles approach each other, and the primary leaf stalk bends downwards (fig. 61).

Interesting studies on the effect of external conditions on irritability have been performed on Mimosa. It has been shown, for instance, that its tonus is affected by temperature, and that this plant passes into cold rigor at low temperatures, and into heat rigor at 40° C. Drought rigor may result from insufficient water, and dark rigor from prolonged absence of light. The power of movement is lost under anaerobic conditions, or in the presence of narcotics (*e.g.*, chloroform). Mimosa, however, again becomes irritable on return to normal conditions, provided it has not continued too long in a state of rigor.

When an insect alights on a leaf of the Venus' fly-trap and touches one of the bristles, a haptonastic response is evoked. The halves of the normal leaf blade fold about the midrib and come together, imprisoning the insect, and a digestive juice is secreted.

Haptonastic movements are also shown by the stamens of certain species belonging to the families Berberidaceae and Compositae, and by the stigmas of the genera Mimulus, Strobilanthes, and others. Thus the stamens of species of Berberis move inwards, and the flaps of the stigmas of species of Mimulus come together, when they are stimulated by contact.

D. Sequence of Events (Perception and Transmission of, and Response to Stimuli) leading to Paratonic Plant Movements

The first link in the chain of stimulation that leads to the performance of a paratonic movement by a motor region is the perception of the effective external stimulus. This implies that an excitation or a disturbance is set up in the living cells where the stimulus is received or nearby. Irritability towards a given stimulus may be distributed over extensive portions of some plant organs : for example, all parts of the root and shoot systems of *Mimosa pudica* appear to be sensitive to the stimulus of injury. We shall, however, point out in the next section that the reception of certain external stimuli may be confined to perceptive regions. These, like the sense organs of animals, often appear to be specialized for the work they have to perform.

Perceptive regions and motor regions are as a rule separated from one another in space. We have therefore to account for the passage to a distant zone of disturbances caused by stimulation. Plainly, either something new is transmitted from the region of perception to

the motor region, or, a process of transmission, which was occurring before perception, occurs in a modified form after perception. Until recently the idea was widely favoured that a new state of excitation was induced during perception and transmitted to the motor region ; and much consideration was given to the nature of this excitation and the mode of its transmission. The nervous system co-ordinates the functional activities of sense and motor organs in animals, but plants do not possess a specialized nervous system. Nevertheless, it was a very suggestive fact that delicate protoplasmic threads traverse the walls between adjoining cells in certain living tissues (*cf.* discussion on p. 349). Certain physiologists have held that excitations may travel along such irritable threads in what was appropriately termed stimulus conducting tissue. The functioning of these threads, however, will not be further considered here, for the subject requires reconsideration in the light of modern knowledge, since for certain plant movements it has now been shown that stimuli may be transmitted from perceptive regions to motor regions across a watery gap between two parts of a severed organ (see sections G, H, and J), and that growth curvatures are controlled not by a special chemical produced by stimulation, but by the distribution of and responses to growth hormones (auxins) present in the responding organ before stimulation.

E. The Separation in Space of Perceptive and Motor Regions

Haptotropism and haptonasticism: Organs which can respond to the stimulus of contact furnish good examples of the spatial separation of perceptive and motor regions. Contact must necessarily be made with the outer surface of the organ, and it appears that in many instances it is the protoplasm in the cells of the epidermal layer which actually perceives the stimulus. Thus, in the epidermal cells of the tendrils of certain species of the Cucurbitaceae, there occur tactile pits, *i.e.*, unthickened areas in the outer walls that are occupied by special disc-like extensions of the protoplasm.[1] These pits render it more probable that a series of successive contacts made by the outer surface of the tendril with a solid surface will cause the irritable protoplasm in the epidermal cells to suffer deformation, and in this way perceive the stimulus of contact. It has also been observed that minute papillose protoplasmic processes project into the outer cell walls of the superficial glandular cells of the sundew's tentacles. The actual reception of the stimulus by the outer wall of the perceptive epidermis is sometimes facilitated by the papillose nature of this wall. For example, such tactile papillæ, but of a specialized kind, are found in the middle part of the filament of each stamen of the barberry. In the Venus' fly-trap, and in species of Mimosa, the association of multicellular stiff tactile bristles with sensitive tissue augments the intensity of the stimulus of contact. The sensitive tissue is found in the basal part of the bristle. Consequently when the tip of the stiff bristle receives the contact stimulus an augmented displacement occurs at the basal end.

[1] For details concerning the structure of perceptive and motor regions see Haberlandt (*109*).

The spatial relations between the perceptive regions and the regions of haptotropic or haptonastic response are various. In the stamens of the barberry the perceptive layer overlies the motor tissue of each filament; and the stimulus does not have to travel far in tendrils such as those of the passion flower, in which the concave side is sensitive and curvature is brought about by accelerated growth on the convex side. Wider separation of perceptive and responding regions occurs in the Venus' fly-trap and the sundew. In the former plant, peculiar sensitivity is associated with the three tactile bristles on each half leaf. Response takes the form of a movement of variation which is governed by the pulvini at the hinge between the half-leaves. In the sundew, growth curvatures of the tentacles follow stimulation of the glandular heads.

It appears that in *Mimosa pudica* the greatest sensitivity to contact is found near the tactile bristles on the pulvini of the main petioles. Contact stimulation leads to a haptonastic movement of variation, not only of the main petiole, but of leaflets and pinnules, and the disturbance may be transmitted over a long distance. The spatial separation of perceptive and motor regions is also well illustrated when injury to a small plant (*e.g.*, singeing the tip of a pinnule) evokes responses in all the pulvini on the plant.

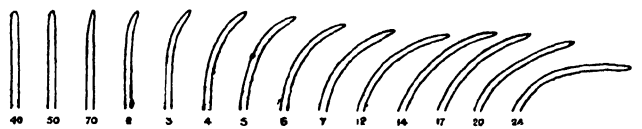


FIG. 63. Successive positions of a coleoptile of oat in the dark at 17·5° C. when rotated on a horizontal klinostat, after receiving light for four seconds from a lamp of 30 metre-candles placed on the right. The numbers give the time after the stimulation, the first three in minutes, and the remainder in hours. (From Arisz, see Kostytschew, *174*.)

Phototropism. Both Francis Darwin and Rothert selected seedling plants of the grass family as suitable material for their pioneer experiments on the spatial separation of perceptive and motor regions in phototropism. The seedling shoot of Setaria consists of a hypocotyl bearing aloft a spherical cotyledon in which the plumule is enclosed. It was shown that the positive phototropism of this shoot, which is normally displayed by the differential growth of the hypocotyl, cannot be evoked by unilateral illumination if the cotyledon is cut off or covered with a cap of tinfoil. It was inferred that in this grass phototropic sensitivity resides exclusively in the spherical cotyledon, and, consequently, that perceptive and motor regions are distinct.

In more recent investigations on oat seedlings, which should be used before the plumule has pierced the coleoptile sheath, F. W. Went found that although the base of the coleoptile may be slightly sensitive, phototropic sensitivity chiefly resides in a length of less than 2 mm. at the tip. Stimulation of the tip induces growth curvatures at successively lower levels in the coleoptile (fig. 63). Thus it

has been inferred that there is a gradual and differential basipetal transmission of something from the perceptive tip to the motor region where the cells are undergoing turgor enlargement.

It is undecided whether phototropic sensitivity is possessed by all or only by a limited number of living cells in the perceptive regions. At one time much discussion centred on Haberlandt's view that papillose forms of epidermal cells are adaptations for the reception of the light stimulus. There is no doubt about their normally acting as condensing lenses that concentrate the incident light on the irritable protoplasm, but it is at present widely accepted that this is not an essential phenomenon in phototropism. Thus phototropism is still displayed when the surface of the organ is smeared with oil, which converts the epidermal cells from convergent to divergent lenses.

Geotropism. Charles Darwin was the first experimenter to show that no geotropic curvatures take place in decapitated roots changed from vertical to horizontal positions. Convincing demonstration presents difficulties as roots show what are termed traumatropic curvature responses to the stimulus of wounding. Easily distinguishable geotropic curvatures can be induced, however, by placing roots in horizontal positions for longer than the presentation time (see section F), and decapitating before any curvature is noticeable. It may be concluded from the two sets of experiments that tips are necessary for the perception of, but not for the response to, the stimulus of gravity, and, consequently, that perceptive and motor regions in the root are distinct. Piccard, however, obtained evidence that growing zones could perceive as well as respond to geotropic stimuli (see Pfeffer (*228*), vol. III, p. 418, and Stiles (*291*), p. 469); but they are less sensitive than the so-called perceptive regions.

It appears from decapitation experiments that the tips of seedling plants of the grass family should be regarded as the geoperceptive regions. Dolk has recently demonstrated that the whole of the oat coleoptile is geoperceptive, but that irritability decreases steadily from the tip to the base.

In order to demonstrate, without injuring roots, that it is the position of the root tip in relation to the line of action of the gravitational force that determines whether geotropic curvatures will occur, Czapek enclosed the tips of seedling roots in glass slippers which were placed either in vertical positions or at various fixed angles to the vertical. He showed that no matter what angle to the vertical was made by the rest of the root, geotropic curvatures did not occur so long as the tip was directed vertically downwards, and that geotropic curvatures always occurred even in vertically placed growing regions, provided the tips were at an angle to the vertical. Experiments of a similar kind and yielding similar results were performed by Francis Darwin on shoots of seedling grasses. He showed that it is the position of a shoot apex which determines whether geotropic curvatures will take place.

There remains to be considered the question of the mechanism whereby gravity affects the irritable protoplasm in geoperceptive regions. It was Noll who first suggested that the presence of movable starch grains in plant cells might have a bearing on this question, and his suggestion has received support from later investigations, particularly those of Němec and of Haberlandt.

It is evident that gravity must act upon substances which either form part of the living protoplasm (such as oil drops, which tend to rise in an aqueous medium) or are metaplastic bodies included in the protoplasm (such as starch grains, crystals of calcium oxalate, silica, etc.). The name statolith has been suggested for these latter substances, the inclusions. We may define a statolith as a visible cell inclusion which will shift its position in the cell under the influence of gravity. The name statocyst has been given to a geoperceptive cell which contains statoliths. It is supposed that the protoplasm in the cells possesses irritability towards the pressure exerted by the statoliths. According to this view, it is the static pressure of statoliths upon the protoplasm lining the lowermost wall of the cell which determines that a main root will normally grow vertically downwards. When the root is displaced from the vertical position, the statoliths will fall against another portion of the protoplasm : " A new and unfamiliar state of stimulation is thereby produced, with the result that a geotropic movement takes place which brings the organ back into its former state of equilibrium." [1] Although statolith starch or other visible grains are absent from the roots of maize and of other plants that can show geotropism, there can be no question but that the " distribution in space and time of geotropic sensitiveness is correlated in a remarkable manner with the presence of falling grains".

It has been demonstrated that cells containing movable visible grains occur in the central part of the root cap of main roots, and in the cells of the coleoptile tip of grass seedlings. Thus the observed distribution of statocysts accords well with the views that are held concerning the position of the geoperceptive regions in these organs. It has been suggested that in stems the cells of the starch sheath, or endodermis, may act as statocysts. Sometimes, however, instead of a continuous layer of statocysts, these functional units are scattered as groups of cells which are associated with vascular bundles, etc. The point to note is that statoliths have been observed in stems, inflorescence axes, peduncles, grass pulvini, petioles, and leaves. Hawker (*119*) has suggested that statocysts are arranged in three zones which she terms (*a*) zones of development, where grains are present but not free to fall, (*b*) zones of efficiency, which function in geoperception, and (*c*) zones of disintegration, where sensitivity has been lost owing to the splitting of big grains.

Experimental evidence in support of the statolith hypothesis of geoperception has been put forward by Němec, Haberlandt, and others. It

[1] The quotations are from Haberlandt (*109*).

has been stated that decapitated roots do not become geoperceptive again until starch grains are formed in the wound callus. Also, it was found that growth ceased and starch grains disappeared from the root caps of radicles of broad beans which had been embedded in plaster of Paris; and that after removing the plaster of Paris the roots started to grow again, but sensitivity towards gravity did not return until starch grains were regenerated in the root cap. Further evidence was afforded by the observation that the adventitious roots first formed by sprouting onion bulbs, which had previously been stored for several years in a dry place, did not give geotropic responses unless starch grains were formed in the root cap.

There are other observations for various organs which might be adduced in further support of the view that movable starch grains or other solid particles may play an essential part in the early events that lead to geotropic response. For example, Haines (*110*) has reported that what is termed plegetropism is displayed as a movement of response of an organ to a change in its velocity, and argued that this must be primarily brought about through the mechanical distribution of particles in the protoplasm. He maintains that this phenomenon provides evidence of the fundamental truth of the statolith hypothesis.

F. Time Relations of Plant Movements

Experiments show that response by movement may be delayed until some time after the inducing stimulus has been removed. Thus, (*a*) coleoptiles of oat seedlings show phototropic curvatures in the dark as after-effects of changes induced during a period of lateral illumination; (*b*) organs may display geotropism, while rotating on a klinostat, provided they have previously been subjected to geotropic stimulation for longer than the presentation time (see below); and (*c*) there is an appreciable interval between haptotropic or haptonastic response and the application of a single contact stimulus. Plainly, perception and reaction are linked events which are separated in time.

In discussions of the time relations of plant movements frequent use has been made in the past of terms such as reaction time, presentation time, and relaxation time. The reaction time is the time taken for a visible response to be shown by an organ placed under constant stimulation. Upon stimulation Mimosa shows movement almost instantaneously. Haptotropic responses also are rapid, being clearly evident in less than a minute after vigorous stimulation. Usually at least half an hour elapses before the geotropic responses of young organs become visible; phototropic responses are usually more rapid. It appears that the reaction time for a given plant will depend upon (*a*) *the amount of stimulation;* thus Arisz showed that when etiolated oat coleoptiles responded in the dark to the stimulation experienced during a single period of lateral illumination, the reaction time increased as the quantity of stimulus was decreased; (*b*) *the external conditions*, such as temperature; (*c*) *the stage of development of the organ and its previous history;* a good example is that plants grown

in the dark react more readily to unilateral light than plants that have been grown under ordinary conditions. It is also evident that if observations are made with a microscope, or if a lever arrangement is used for amplifying angular divergences from the main axis, the observed time of reaction will be considerably shortened.

It is probable that the perception of a stimulus takes place instantaneously, *i.e.*, some change occurs in the irritable member immediately the stimulus is received. Visible response, however, requires that the stimulus must be received in excess of a certain minimal quantity. The presentation time is the time for which a given member must be continuously subjected to a given intensity of stimulus, for a visible response to follow inevitably. When the geotropic stimulus is acting to its full extent on young stems the presentation time is just over five minutes. Plants should be placed so that the organs under experiment are in a horizontal position on a horizontal intermittent klinostat. Comparable samples should be used to determine what is the shortest period for which the organs must be left in a horizontal position when the klinostat is at rest, for a subsequent curvature to be shown when the klinostat is rotated (fig. 62, c).

The intensity of the geotropic stimulus may be varied by altering the angle between the irritable organ and the line of action of gravity. Experiments indicate that for a given member, the presentation time is inversely proportional to the effective force with which gravity is acting on the irritable member. The same relation holds when centrifugal force is substituted for gravity. Thus for a centrifugal force of 3 units acting on oat seedlings, the presentation time was 100 seconds, but on increasing the force to 58 units, the presentation time was reduced to 5 seconds. In general, these experiments indicate that.

$$\text{Centrifugal force} \times \text{Presentation time} = \text{Constant.}$$

More recent work shows that a comparable product law governs the relations between intensity of light and the presentation time in phototropic responses. It is the quantity of light, which is compounded of intensity and duration of illumination, which determines the amount of the response (see p. 403). When a strong source of light is used for a very sensitive organ, a single flash may be sufficient to evoke a response. Clearly under these circumstances the concept of presentation time has little value.

Experimental studies on presentation time led to others on the effects of stimulations, which, though in themselves insufficient to evoke responses, may nevertheless cause disturbances that persist. Thus, if a root is left in a horizontal position on an intermittent klinostat, which is at rest for a period that is shorter than the presentation time, no curvatures will occur on subsequent rotation on the klinostat. If, however, after a period, the

klinostat is stopped and the root is again exposed to the unilateral influence of gravity for another period shorter than the presentation time, a geotropic curvature may be executed after rotation is renewed (fig. 53, *c*); or several such intermittent stimulations of duration less than the presentation time may be necessary to evoke geotropism. The response is induced as a result of a summation of stimuli, each of which alone is insufficient to induce a geotropic curvature. The quantitative relations of this cumulative effect of disturbances are complex. For instance, if stoppage and rotation of the intermittent klinostat alternate sufficiently frequently, the total time of intermittent stimulation is in the sum approximately equal to the presentation time, while with longer rotations between the stoppages the sum of the times of intermittent stimulations becomes considerably greater than the presentation time. This means that a disturbance not sufficiently great to evoke a response may subside if the organ is no longer being stimulated, and many experiments of the kind we have discussed have been performed to measure the relaxation time (the time taken for a stimulation to subside) for geotropism.[1]

G. The Parts Played by the Light-growth Reaction and by Auxins in Phototropic Responses [2]

About a century ago de Candolle suggested that positive phototropic curvatures resulted from the partial etiolation, and hence from the enhanced growth, of the shaded side of a growing member illuminated from one side only. But this suggestion did not prove acceptable as it offered no explanation of the negative phototropism displayed by certain roots (*e.g.*, white mustard, aerial roots of ivy) and branch tendrils (*e.g.*, vine, Virginian creeper). Wolkoff, however, maintained that negative phototropism might also be explained by de Candolle's etiolation hypothesis. He suggested that, owing to the refraction of the incident rays, and their subsequent focusing, the tissues of the shaded side of an organ may, under certain conditions, be more strongly illuminated than those of the side next to the source of light. The growth rate of the near side would, therefore, be retarded less than that of the distant side, and a negatively phototropic curvature would result. Wolkoff's idea was later described as " quaint," and dismissed as being " totally incorrect." [3] For many years no further attempt was made to analyse the mechanism of phototropic response. It was accepted as a fact that a structure irritable towards light can appreciate certain differences in intensity on opposite sides and will, according to its inherent nature, respond positively, negatively, or transversely. Interest then became centred on the laws governing the minimum differences of intensity that induced phototropism, *i.e.*, the threshold value of the stimulus, for different combinations of light intensity. Certain authorities (*e.g.*, F. A. F. C. Went) have expressed doubt whether great profit has attended researches in this field, and since Blaauw began his pioneering researches interest has largely been transferred to other and more fruitful studies.

After Blaauw had ascertained the light-growth reactions of certain plant members (p. 355) he made investigations on phototropic

[1] For a discussion of the biological interest of the idea of summation of stimuli, see Darwin, F. (*70*).

[2] A comprehensive and critical review of old and modern experiments on and theories concerning phototropism and allied phenomena of growth has been written by du Buy and Nuernbergk (*58*).

[3] Pfeffer (*228*), vol. III, p. 229.

curvatures. The plant was grown in the dark, and, after a while, a quantity of light, measured in metre-candle-seconds (m.c.s.), was allowed to fall on one side only of the member under observation. Subsequently the plant was again left in the dark. Phototropic growth-curvature occurred as an after-effect and the maximum angle made with the vertical was measured. Blaauw made the fundamental discovery that it is the quantity of light received that determines the magnitude of the reaction. Thus a quantity Q, received from a low intensity over a long time, evoked the same reaction as the same quantity Q received from a high intensity for only a short time. Different quantities of light brought about different degrees of curvature, showing that the once-cherished idea of " all or none " for responses to stimuli has no warrant for phototropism.

Further, Blaauw was able to confirm earlier observations that the nature of phototropic response is not invariable for a given organ. For instance, he discovered by his quantitative single-illumination method that it is the quantity of light received that determines the reaction of the sporangiophore of *Phycomyces*. As the quantity was increased, he found that the reaction changed from positive to negative and then to a second positive. Arisz later demonstrated a similar variability for the coleoptile of the oat, and for this structure de Buy has recently obtained evidence of a third positive reaction.

Blaauw came to the conclusion from naked-eye observations that the threshold quantity of stimulus for the oat coleoptile was about 20 m.c.s. Arisz also succeeded in equating the amount of curvature with the quantity of stimulus. He used a microscope to detect small deviations of the coleoptile tip from the original vertical position, and observed that even 1·4 m.c.s. could cause curvatures. There is no reason to suppose that still smaller quantities of unilateral light may not, in spite of abstract notions about threshold values, cause reactions which have not yet been detected. Apparently, however, for small quantities of light, it is extremely difficult to distinguish between phototropic and autonutational curvatures. It may well be, therefore, that over a certain range the quantity of stimulus (measured by the product of intensity and time) determines the amount of response. It should be realized, however, that the stimulus does not provide the energy for the response. This, presumably, comes from respiratory processes, and is far in excess of the energy introduced by the stimulus (*cf.* p. 4).

To explain phototropic curvatures Blaauw returned to de Candolle's ideas, but he based his theory on his own experiments (p. 356) on the light-growth responses of different organs. For stems (*e.g.*, the hypocotyl of the sunflower seedling) he, like de Candolle, attributed

the positive phototropism to the negative growth reaction towards light. Blaauw was able to meet the earlier objections to the etiolation hypothesis after he had found that, whereas the roots of the oat and the radish showed no light-growth response and did not curve under lateral illumination, those of the white mustard, which had long been known to give a negative phototropic reaction towards continuous unilateral daylight, exhibited a distinct light-growth reaction. It should be noted that photosensitivity of roots, as illustrated in certain botanical books, is the exception and not the rule.

Blaauw studied the behaviour of the sporangiophore of Phycomyces, and found that light caused an acceleration of the growth rate of this organ, *i.e.*, the total light-growth reaction of this organ was positive (*cf.* the effect of light on stems). Consequently, unless some additional factor has to be taken into account, one would expect this organ to show negative phototropism. For certain quantities of light it actually gave negative phototropic responses, but for others it displayed positive phototropism. To explain this Blaauw returned to Wolkoff's idea that the transparent sporangiophore exerts a lens-like action on such incident light as is not dispersed. By using photographic paper he educed some evidence that light is focused on the distant side of the sporangiophore, and concluded that, as a result of the focusing, the growth rate on the distant side is greater than on the near side, *i.e.*, a positive curvature is induced.

Van Dillewijn's quantitative researches have in recent years provided strong support for Blaauw's contention that light-growth reactions play a part in determining the amount and the nature of phototropic curvatures. Using photographic paper he concluded from his experiment that only one-thirtieth of the incident illumination reached the distant side of the oat coleoptile. The remainder of the light was absorbed and dispersed. He also determined the light-growth reactions for this coleoptile under quantities of uniform illumination (p. 357) ranging from quite small values to over 100,000 m.c.s. The results enabled him to calculate what the growth rates on two receiving sides of a coleoptile would be subsequent to illuminating with a given quantity of light from one side only (*i.e.*, receiving Q m.c.s. on the near side and Q/30 m.c.s. on the other), and so to predict the extent of the angular divergence from the vertical, which gives a measure of the phototropic response. His predictions were found to be justified, for his calculated results tallied within the limits of experimental error with the results of his own and of Arisz's earlier experiments on phototropism.

Blaauw's theory of phototropism may be summed thus : *the light-growth reaction is the primary, phototropism is the secondary phenomenon which follows of necessity.* The implication is that unilateral illumination has a direct differential effect on the enlarging regions of

the sensitive organ. But we have already seen (section E) that in the grass family, for example, the stimulus of light is mainly perceived by the cotyledon (Setaria) or the tip of the coleoptile (oat). It appears, however, that light has a direct effect on the enlarging cells of the coleoptile, which shows slight sensitivity over its entire length (see also p. 358). It became clear that Blaauw's theory required modification; and in 1927 F. W. Went published the results of ingenious quantitative researches which substantiated Boysen-Jensen's and Paál's earlier suggestions (see p. 365) that phototropism is governed by the behaviour in illuminated regions of the growth substances now known as auxins.

F. W. Went first showed that the highly perceptive tip is rich, and the rest of the oat coleoptile, which is but slightly photosensitive, is poor in auxin. He also found that a coleoptile without a tip again becomes sensitive to light two hours after decapitation, *i.e.*, after the regeneration of a new physiological tip (p. 367). He then set out to investigate the effect of unilateral light upon the distribution of auxin in the tip. He cut off tips from coleoptiles that had received 1,000 m.c.s. of light from one side only, and placed them on standard agar blocks with a safety razor blade between the sides that had been near to and distant from the light source. The half-blocks were then placed eccentrically on different decapitated coleoptiles in the dark. Growth curvatures were executed and the angle of divergence was measured in the usual way. The mean of several experiments pointed to a destruction or annulling of sixteen per cent. of the growth stuffs in whole tips as a result of unilateral illumination with 1,000 m.c.s. (*cf.* the destruction measured in the experiments (see p. 359) on coleoptiles under uniform illumination). Of the residual eighty-four per cent. of the growth stuffs, fifty-seven per cent. was present in the half-tip from the dark side, and twenty-seven per cent. in the half-tip from the illuminated side. Control experiments showed that the auxin was uniformly distributed in tips of coleoptiles which had been kept in the dark. Went concluded that one-sided illumination occasions a lateral movement of auxins to the side distant from the source of light, and, consequently, that more auxin will be translocated downwards to and be received by the enlarging cells on this side than by the cells on the illuminated side. Seeing that the rate of growth is governed by the amount of auxin received by the enlarging cells (p. 368), greater growth would occur on the shaded side, and positive phototropism would be displayed. *Went's theory, therefore* (cf. *Blaauw's*, p. 404), *attaches first importance to the redistribution or polarization of auxin.*

In 1932 van Overbeek (*220*), as a result of his researches on the light-growth reactions (p. 359) and the phototropism of Raphanus seedlings, advanced the view that Blaauw's and Went's ideas should be com-

bined in the interpretation of phototropism. He first confirmed Went's view by showing that auxin is preferentially drawn to the shaded side of the perceptive regions of the seedlings of Avena and Raphanus when these are laterally illuminated. He then experimented with pieces of hypocotyl of Raphanus, each of which he covered with a block of agar containing auxin. The basal ends were placed on a safety razor blade so as to divide each hypocotyl into two equal parts. A separate block (A and B) of pure agar was placed under each part of each hypocotyl, and the auxin content of these blocks at the end of an experiment was taken as a measure of the relative amounts of auxin that had travelled down the two sides of the hypocotyl from the covering block. Van Overbeek found that at the end of the experiment in the dark, equal amounts of auxin had collected in the blocks A and B, but when one-sided illumination had been used (fig. 64) the amount of auxin in the block B was nearly twice as great as that in the block A. Evidently unilateral illumination had caused unequal distribution of auxin in the conducting region. Hence not only in the regions of perception—as Went first proved—but in stimulus conducting tissue auxin is drawn to the shaded side. This fact is bound to prove of importance in further work on phototropism. *Here we note that it provides further support of Went's contention that phototropism is produced as a result of the unequal distribution of auxin.*

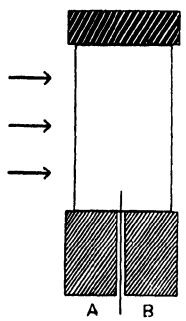


FIG. 64. The effect of unilateral illumination from the direction indicated by the arrows on the distribution of auxin in the conducting region of Raphanus (see text).

In the early stages of the investigation of phototropism at Utrecht importance was attached to Went's discovery that light (1,000 m.c.s.) annulled or destroyed about twenty per cent. of the auxin in the tips of oat coleoptiles, and further evidence of such inactivation has been obtained by some recent experiments (p. 359). Koningsberger and Verkaaik (*173*) made experiments on illuminated and darkened coleoptiles, which had been twice decapitated. The stumps were topped with auxin-a (supplied by Kögl) or with synthetic heteroauxin, and results were obtained similar to those reported by van Overbeek, *i.e.*, they obtained photoretardation of growth when auxin-a was used, but not with heteroauxin. In an important additional experiment they exposed for five hours to unilateral illumination with 100 metre-candles, coleoptile stumps covered *centrally* with plain agar in some experiments, and in others with agar containing equal physiological concentrations either of auxin-a or of heteroauxin. In the experiment with plain agar the phototropic curvatures were insignifi-

cant; this, of course, could be attributed to the inability of the coleoptiles to grow owing to the lack of growth hormone. Strong positive curvatures were observed in the experiments with auxin-a, such as have so often been obtained under like conditions. But no curvatures were shown in the experiments with heteroauxin, although it is known that this substance has strong growth promoting properties. If the coleoptiles showed straight growth it may be inferred that in spite of unilateral illumination the heteroauxin did not move towards the shaded side during its basipetal migration. Inasmuch as heteroauxin is photostable and as auxin-a undergoes photo-inactivation (probably by means of its lactone), Koningsberger and Verkaaik *attributed the positive phototropic curvatures shown by the basal regions of coleoptiles in their experiments with auxin-a to the partial inactivation of this hormone on the illuminated side of the coleoptiles.* Their results did not appear to them to give any support to Went's theory of a redistribution of auxin-a owing to lateral transfer.

It is recalled that van Overbeek reported that in Raphanus seedlings the auxin content of the cotyledons and the tips of hypocotyls did not diminish when they were illuminated, and in considering the *direct effect of light* (Blaauw's theory), he attached great importance to his discovery that, in spite of this fact, the growth response of a hypocotyl of Raphanus to a given quantity of auxin is less in light than in the dark (p. 360). He concluded that light reduces the stimulating influence auxin exerts on the extensibility of the cells of the enlarging region. He states, " In the dark the cells are more sensitive to growth substance than in the light. In hypocotyls exposed from one side, it is therefore very probable that the shaded side is more sensitive to growth substance than the illuminated side. Even when the growth substance is equally distributed and the shaded side has a greater sensitivity to growth substance, the hypocotyl will grow towards the light. *It seems to me that there is a resemblance between the principle of Blaauw's theory of the light-growth reaction and the phenomenon of the greater sensitivity of the cells to growth substance in the dark than in the light.*"

It is evident that no theory of phototropic mechanism in terms of auxin control can at present be put forward to cover all the known facts about the behaviour of sensitive structures. What may be true for oat coleoptiles does not appear to be necessarily true for the seedling shoots of Raphanus. Furthermore, it must be remembered that the sporangiophore of Phycomyces does not contain auxin-a, and that heteroauxin, which may be present in this fungus, is said to be photostable. It is clearly possible that in some instances phototropic curvatures may result from chemical or physical changes in which auxins and similar substances do not participate.

H. The Part played by Auxins in Geotropic Responses [1]

Cholodny has proposed a theory in which he attributes the differential growth rates that lead to the geotropic curvatures of stems and roots placed in a horizontal position, (*a*) to the greater accumulation, owing to oblique travel, of auxins in the lower sides of the elongating zones, and (*b*) to the inherent differences in the responses of stem and root to auxins. We shall refer below to Dolk's work at Utrecht in support of contention (*a*). First, however, let us recall the results of Cholodny's experiments, which, as was stated on p. 368, have been confirmed by Keeble, Nelson, and Snow. A coleoptile tip of maize (*i.e.*, the geoperceptive region of this organ), or an agar block containing auxin, retards the growth rate of a decapitated maize root. This contrasts strikingly with the accelerating effect of auxin on decapitated coleoptiles, and supports Cholodny's contention (*b*) above. Further support was derived from experiments which showed that whereas root tips retard the growth rates of decapitated roots on which they are stuck, they accelerate the growth rates of decapitated coleoptiles. Hawker has demonstrated that shoot and root differ in their responses to auxin. She found that fixing root tips of the broad bean eccentrically upon decapitated roots induced growth curvatures in the direction of the covered side. This contrasted with F. W. Went's experiment in which it was shown that decapitated coleoptiles curved away from the side covered by tips or agar blocks containing auxin.

It is clear from the foregoing evidence that if auxin becomes more concentrated on the lower than on the upper sides of horizontally placed organs, growth curvatures will inevitably occur, and that those performed by a shoot organ will be away from the lower side (negatively geotropic) and those by the root towards the lower side (positively geotropic).

We owe to Dolk's work the important information that in some way or other as yet not known, gravity causes a redistribution of auxin in the tip of an oat coleoptile placed in a horizontal position. This redistribution leads, as is required by Cholodny's theory, to the gathering of auxin in relatively greater concentration on the lower side of the tip. Dolk first ascertained the presentation time for oat coleoptiles. He then decapitated coleoptiles that had lain horizontally for longer than this time, and placed the tips on agar, separating by a safety razor blade the upper from the lower half of each tip (*cf.* F. W. Went's experiment on phototropism). The agar blocks that had been under the half-tips were subsequently placed eccentrically upon headless vertical coleoptile stumps; unequal growth occurred, and from the resulting angles of curvature the

[1] See Snow's review (*137*).

relative concentration of auxin in each block and hence in each half-tip was computed. The concentration of auxin in the half-tips that had been lowermost appeared always to be greater than that in the half-tips that had been uppermost.

An important point shown by Dolk was that neither the rate of growth nor the total amount of auxin present in coleoptiles was altered by changing these organs from a vertical to a horizontal position. The effect of gravity as an external stimulus upon auxin is, therefore, different from that of light. There is no gravity-growth reaction similar to the light-growth reaction demonstrated by Blaauw. It appears that geoperception leads to a polarization of auxin in apices, and it may be inferred that this state of polarization gradually travels from the perceptive regions and induces curvatures in the elongating regions of the shoot and root, those regions nearest the perceptive tips being the first to react. It is supposed that auxin either directly or indirectly brings about an increase in the plasticity of cell walls of coleoptiles (p. 373), and that this accounts for the differential growth rates in these structures. To bring about the opposite tropic curvature in roots, the auxin may either have a different effect upon cell walls, or alter the permeability or some other property of cells that are capable of expansion. Experimental evidence which might throw light on this obscurity is as yet lacking. It appears, however, that negative geotropism is correlated with an accelerating influence of auxins upon the growth of shoots and positive geotropism with a retarding influence of the same or closely similar auxins upon that of roots. It remains to be seen whether Cholodny's theory will explain the plagiotropism towards gravity of secondary roots, lateral branches and leaves, and of changing responses during development (p. 390) or through correlative adjustment to injury in another organ (p. 390). It may be, as certain investigators have suggested, that responses to gravity are not wholly tropic, and that work on the supposed nastic component of the total response will provide answers to some of the outstanding questions.

I. Auxins and the Autotropic Reversals Following Certain Phototropic and Geotropic Curvatures

In the experiment illustrated in fig. 60 it is seen that geotropic curvature of the stem does notstop when the stem has reached the vertical position. The over-curvature is, however, followed by a straightening. This has for a long time been called an autotropic reversal. Since organs show this reversal by straightening, after undergoing geotropic curvature on a klinostat, following previous stimulation by gravity, it follows that not gravity but an internal state of affairs governs the straightening. Dolk showed that during rotation on a klinostat the distribution of previously polarized auxins speedily became equalized again. This would account for the stoppage of the movement of curvature but not for reversal. Similarly, F. W. Went found that the phototropic curvature in the dark subsequent

to a single lateral illumination was followed by an autotropic straightening, and this he correlated with the regeneration of auxin in the tip. This regeneration would account for the cessation of phototropic curvature but not for autotropic reversal.

Dolk has expressed agreement with Went's explanation of autotropic reversals. Went pointed out that during growth curvatures more cell-building materials (presumably foodstuffs) are being used on the side growing more rapidly. An excess would thus be left on the other side. When growth stuffs again become equally distributed, the side previously growing less rapidly would grow more rapidly because it would possess residual foods in greater concentrations than the other side. Autotropic reversal would, therefore, continue until the concentration of foodstuffs as well as that of auxin was equalized all round the growing structure.

There appears to be no reason why this simple hypothesis should not cover other straightening reversals following nastic curvatures and other growth movements.

J. The Hormone Theory of the Transmission of Stimuli in Sensitive Plants [1]

The transmission of stimuli in sensitive plants is supposed to be the most rapid of all forms of stimulus transmission that occur in plants. The rate depends upon the mode of stimulation and the state of the plant. Measurements have been made on the assumption that visible response takes place immediately the stimulus arrives at the pulvini, and variations in speed from 2 to 15 millimetres per second have been recorded.[2]

The notion of Sir Jagadis Chundra Bose that transmission is effected by the propagation of protoplasmic excitations along a sort of nervous system in plants was at no time generally accepted by physiologists, and has in recent years been supplanted by another theory. Using exquisitely delicate physical methods, he has, however, collected a valuable mass of data, which, doubtless, will in time be interpreted in the light of modern knowledge (Bose, *41*).

It had long been known that stimuli could be transmitted along ringed stems (Dutrochet, 1824), and lengths of stem which had been killed by exposure to the temperature of boiling water. Since, however, the mode of transmission appeared to differ from that in whole living plants, the possibility that under natural conditions living cells played some part in transmission could not be excluded. The problem has again been investigated in more recent years. Ricca removed a ring of all tissues external to the wood and bored out the pith from a cut stem of a species of Mimosa, and found that the plant still responded to stimulation and that transmission took place at about the same rate as in whole plants. He inferred that under normal conditions the stimulus is transmitted through the wood.

[1] For an account of recent work see Ball (*11*).

[2] *Cf.* the estimates of speeds of the order 30,000 millimetres per second, which have been made for the transmission of stimuli in the nerves of higher animals.

He next severed a stem of a whole plant and connected the rooted stump and cut shoot by means of a rubber tube filled with water, and made the discovery that the stimulus could be transmitted across the discontinuity through the watery medium. Ricca's experiments have been confirmed and extended by Snow and by Ball, and it appears to be rigidly established that transmission, differing not a whit from normal transmission, can occur from cut shoot to stump or in the reverse direction across a watery gap, that is in the complete absence of living cells. The conclusion was therefore drawn that transmission is effected by the movement of a chemical substance. As this substance arouses pulvini to activity it was designated by the term hormone, which was taken from the nomenclature of animal physiology.

Preliminary experiments have indicated that this hormone is thermostable in the plant (*cf.* the earlier experiments on transmission along killed stems), but is thermolabile in watery extracts. It is not precipitated by lead acetate, and does not give protein reactions. It can diffuse through a collodion filter.

The present view is that this hormone is liberated at the point of stimulation, and, in normal conduction (which is the only form that occurs when the turgor is low and tensions in the tracheal elements high), is transported to the pulvini that it stimulates in the water current which moves in the xylem. Both Snow and Ball have distinguished between this normal conduction and a more rapid conduction, which is supposed to occur when cells are in a state of maximum turgor. It has been suggested that in this rapid conduction the hormone, when released, causes the contraction of neighbouring cells, probably in the pith. In collapsing, these highly turgid cells eject more hormones, and so by a relay mechanism, a rapid transmission takes place, which is independent of the movement of the water current. It appears that only the pulvini of the main petioles are affected by this rapid conduction, for several experiments indicated that even under conditions favouring high turgor the reaction times for secondary petioles and leaflets were of the relatively slower order characteristic of normal conduction.

APPENDIX I

Notes on the Chemistry of Metabolic Products

THE principal metabolic products mentioned in the main body of this book will be classified in this appendix, on the basis of their chemical affinities. It will be seen that products, originating in different tissues (see chap. XI, section C) and playing very different parts in the life of a plant (see chap. XI, section D), may belong to the same chemical group of substances. Derivatives belonging to the isoprene group provide excellent examples. Although the number of individual substances produced by plant metabolism is exceedingly great, it is possible to sort these substances into a relatively small number of groups. Even within the groups the actual number of compounds found in plants, although sometimes immense (*e.g.*, in the proteins), is usually far less than the number chemists can synthesize *in vitro*. For example, eight pairs of aldohexoses are known to chemists, but only three aldohexoses are produced by protoplasm. Similar restrictions of anabolic powers are encountered in all the groups. This is a noteworthy fact, for it bears the implication that metabolism in plants is directed along a limited number of main lines (see chap. XI, section F).

The book of Haas and Hill (*108*) has for some years been a standard work on this subject, and Steele (*281*) has written a book for botany students on the chemistry of plant products. The experiments described by Onslow (*216*) serve to illustrate the principal chemical properties of metabolic products. In order to define problems of metabolism it is clearly a prior essential that the molecular structure of metabolites and metabolic products should be elucidated. This elucidation is the task of the organic chemist. His achievements have been remarkable not only in the field of analysis, but also in that of synthesis. The reader will find in the English edition of the textbook of Organic Chemistry by Schmidt (*261*) clear accounts of the chemistry of most of the substances mentioned in this appendix.

PART I. ORGANIC COMPOUNDS FREE FROM NITROGEN

The majority of the metabolic products belonging to this class contain only carbon, hydrogen, and oxygen. Occasionally, however, metallic salts of organic acids, and esters of phosphoric acid are found, and, in a few compounds, sulphur is linked to carbon or to oxygen atoms.

A. Hydrocarbons and their Hydroxyl Derivatives

Aliphatic hydrocarbons and their hydroxyl derivatives. (i.) *Hydrocarbons.* Extremely small amounts of ethylene are produced during the ripening of certain fruits (p. 212) ; otherwise there is no definite evidence that the lower members of the paraffin and olefine series of hydrocarbons are produced in the free state by the aerobic metabolism of green plants. The presence among plant products of semi-solid higher homologues has,

however, been reported. Sando (*258*) has separated the higher paraffin, triacontane, $C_{30}H_{62}$, from the wax which covers the cuticle of the apple. Traces of hydrocarbons have been found in the waxes from other plants; for example, Chibnall found nonacosanes in the wax he extracted from leaves of the Brussels sprout.

Alkyl groups (methyl, ethyl, propyl, isopropyl, the butyl and amyl radicals, etc.) and ethylenic linkages, are probably present in all living cells. Derivatives of the acetylene series of hydrocarbons have not been detected in plants.

(ii.) *Alcohols.* The lower members of the series of monohydric saturated alcohols are not normally found in the free state in plants. Ethyl alcohol, however, accumulates in many plant tissues under anaerobic and certain other abnormal conditions (see pp. 264, 287). Some aliphatic alcohols—for example, methyl alcohol, CH_3OH, ethyl alcohol, C_2H_5OH, and amyl alcohol, $C_5H_{11}OH$,—occur in combination with acids as esters. It is noteworthy that both chlorophyll *a* and chlorophyll *b* contain a methyl radical in ester combination. Higher members of the paraffin alcohols also occur in combination as esters. Thus carnaubyl alcohol, $C_{24}H_{49}OH$, is found combined in the wax-like covering of certain leaves. Sando (*loc. cit.*) gave the name malol to the alcohol, $C_{30}H_{48}O_3$, which he discovered among the constituents of apple wax. He also detected the presence of dimyristyl carbinol, $C_{27}H_{56}O$, in this wax.

Allyl alcohol, $CH_2 : CH.CH_2OH$, a monohydric primary unsaturated alcohol, may be a precursor in plant metabolism of allyl sulphide $(C_3H_5)_2S$, the pungent constituent of garlic and of field pennycress, and of allyl isothiocyanate, C_3H_5NCS, which occurs as a glucoside in the seeds of black mustard. Cinnamic alcohol (p. 414) is a phenyl substitution derivative of allyl alcohol.

Dihydric alcohols (glycols) have not been detected in plants, but certain of their oxidation products play an important part in plant metabolism. The first member of the series is called ethylene glycol, $CH_2OH.CH_2OH$.

Glycerol, $C_3H_8O_3$ or $CH_2OH.CHOH.CH_2OH$, a trihydric alcohol, contains one secondary and two primary alcoholic groupings. It does not occur in the free state in plants, but is found combined with acids in fatty oils and lecithins, and is therefore represented in all living cells.

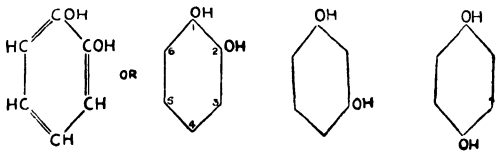
Some of the more complex alcohols are reduction products of the monosaccharides. For example, mannitol is a constituent of manna, celery, asparagus, cauliflower, carrot, etc.; and sorbitol is present in ripe mountain-ash berries and in the fruits of several other genera of the Rosaceae.

Aromatic hydrocarbons and their hydroxyl derivatives. (i) *Hydrocarbons.* Benzene, C_6H_6, does not occur in the free state in plants, but hydrocarbons containing benzene residues are known. Thus styrene, $C_6H_5.CH : CH_2$, occurs in storax; and para-cymene, $C_6H_4.(CH_3).(CH.(CH_3)_2)[1 : 3]$,[1] is found in oil of thyme and in eucalyptus oil. The molecular structure of *p*-cymene is closely related to that of the terpenes (see below).

(ii) *Phenols.* The compounds formed by substituting a hydroxyl group for a hydrogen atom in the benzene ring are called phenols. Thus ordinary phenol is monohydroxy-benzene, C_6H_5OH. Three isomeric dihydroxy-

[1] In writing chemical formulæ we shall place in curved brackets the symbols representing such atoms or radicals as have substituted hydrogen atoms attached to carbon atoms *in* the benzene ring. In order to illustrate this procedure, drawings of the graphic formulæ of the dihydroxy- and trihydroxy-phenols are given later in the text.

phenols are known,[1] viz., catechol or ortho- or 1 : 2-dihydroxy-benzene, $C_6H_4(OH)_2[1:2]$; resorcinol or meta- or 1 : 3-dihydroxy-benzene, $C_6H_4(OH)_2$ [1 : 3]; and quinol or para- or 1 : 4-dihydroxy-benzene, $C_6H_4(OH)_2$ [1 : 4] :—



Catechol. Resorcinol. Quinol or hydroquinone.

Phloroglucinol and pyrogallol are trihydroxy-phenols :—



Pyrogallol
1 : 2 : 3-trihydroxy-benzene
$C_6H_3(OH)_3[1:2:3]$.

Phloroglucinol
1 : 3 : 5-trihydroxybenzene
$C_6H_3(OH)_3[1:3:5]$.

Ordinary phenol is of interest, as it is often used as an antiseptic in biochemical experiments. It does not occur in the free state in plants. The polyhydroxy-phenols are widely distributed in the free state (*e.g.*, quinol occurs in the leaves and flowers of the cowberry), or in glycosidal combination. Catechol and pyrogallol groupings are represented in the tannins. Phenolic derivatives of the homologues of benzene are also widely distributed. For instance, thymol, $C_6H_3.(C_3H_7)\ (CH_3)\ (OH)$ [1 : 2 : 5], occurs with cymene in oil of thyme, and imparts fragrance to this essential oil.

(iii) *Aromatic alcohols.* In the homologues of benzene, hydroxyl groups may also be substituted for hydrogen atoms attached to carbon atoms in the side chain. For instance, by introducing a hydroxyl group into toluene, $C_6H_5.CH_3$, we may obtain either a phenolic substance, viz., cresol (*o*-, *m*- or *p*-), $C_6H_4(OH).(CH_3)$, or a primary alcohol, viz., benzyl alcohol, $C_6H_5.CH_2OH$. Cinnamic alcohol, $C_6H_5.CH:CH.CH_2OH$, is another well-known aromatic alcohol. Saligenin, $C_6H_4(OH).(CH_2OH)$ [1 : 2], *o*-hydroxy-benzyl alcohol, is both a phenol and an alcohol. It is represented in the glucoside, salicin, which is found in the genus Salix. Coniferyl alcohol, $C_6H_3(OH)(OCH_3)(CH:CH.CH_2OH)$ [1 : 2 : 4], a derivative of cinnamic alcohol, occurs as a glucoside, coniferin, in the bark of conifers. Vanillic alcohol,

$$C_6H_3(OH).(OCH_3).(CH_2OH)\,[1:2:4],$$

affords another example of a phenolic alcohol containing a methoxyl group. Derivatives of coniferyl alcohol or of cinnamic alcohol may serve as the building-stones from which the giant molecules of lignin are constructed. Synthesis may be effected by the condensation of alcoholic and phenolic groups with the elimination of water, ether linkages being formed. We

[1] It should be noted that positions 1 : 2 and 1 : 3 in the benzene ring are identical with 1 : 6 and 1 : 5 respectively.

may represent the condensation of two molecules of hydrated caffeic alcohol, a derivative of cinnamic alcohol, thus :

$2\ C_6H_3(OH)_2.CH_2.CH(OH).CH_2OH$
$\rightarrow C_6H_3(OH)_2.CH_2.CH(OH).CH_2{-}O{-}C_6H_3(OH).CH_2.CH(OH).CH_2.OH.$

Evidently the number of condensations taking place in the production of the lignin molecule will determine its size.

Reduced benzene derivatives. Benzene and its homologues may be partly or wholly reduced by the addition of hydrogen. Complete reduction of benzene would yield the saturated ring compound, hexahydrobenzene, C_6H_{12}. Inositol, $C_6H_6(OH)_6$, hexahydroxy-hexahydrobenzene, is an important substitution derivative of this compound. Although inositol is isomeric with the hexose sugars and possesses a sweet taste, it is not a carbohydrate but a polyhydric alcohol. It is widely distributed in plants. In certain seeds inositol is found combined as phytin. Phytin is probably an acid calcium and magnesium salt of inositol-hexaphosphoric acid, $C_6H_6(H_2PO_4)_6$.

Isoprene derivatives. Isoprene C_5H_8, $CH_2 : C\begin{matrix}\diagup CH_3 \\ \diagdown CH : CH_2\end{matrix}$, or,

for economy of space, $CH_2 : C(CH_3).CH : CH_2$, is a substituted di-olefine, which does not occur in the free state in plants, but is set free by the decomposition at high temperatures of certain plant products, *e.g.*, rubber, turpentine. Residues of the isoprene molecule are found linked to one another or to other molecular structures in many important open-chain and cyclic metabolic products. It is not necessary that isoprene should itself be formed as an intermediate metabolite in the production of these compounds. It may well be that molecules containing two or three carbon atoms (such as acetaldehyde, pyruvic acid, etc.) become condensed in various ways, and then reduced, decarboxylated, etc., so as to produce complex substances, which appear to be constructed from isoprene units. Evidently a similar type of metabolism underlies the production of the various substances to be discussed in this subsection. It should be noticed that there is considerable diversity of functional significance among these substances.

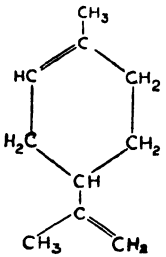
(i) *Terpenes and terpene resins.* Isoprene residues are represented in the molecular structure of the essential oils [1] known as terpenes. As examples of *open-chain olefinic alcohols and aldehydes* we may cite geraniol, $C_9H_{15}.CH_2OH$, and geranial, $C_9H_{15}.CHO$; and citronellol, $C_9H_{17}.CH_2OH$, and citronellal, $C_9H_{17}.CHO$. The structural formula of citronellal,

$$CH_2 : C(CH_3).CH_2.CH_2{-}CH_2.CH(CH_3).CH_2.CHO,$$

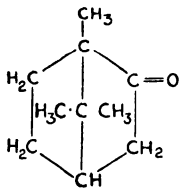
will serve to show how isoprene units may be represented in the combined form in these compounds. The monocyclic terpenes are mainly reduced benzene derivatives. Complex cyclic terpenes are also well known. The naturally occurring *monocyclic and complex cyclic terpenes* may be hydrocarbons (*e.g.*, pinene and limonene), alcohols (*e.g.*, menthol), or ketones (*e.g.*,

[1] The essential oils comprise those liquid components of plant cells which, like the fatty oils (see section C), are immiscible with water, but, unlike the fatty oils, are volatile in steam. They are the scented oils of plants. From the chemical standpoint this group of compounds is heterogeneous. In addition to the terpenes, it includes many aliphatic and aromatic esters ; phenols and aromatic alcohols and aldehydes ; substituted benzene hydrocarbons (cymene, *p*-methyl-isopropyl-benzene) and the mustard oils.

camphor). All these cyclic compounds appear to be constructed from isoprene units.



Limonene,
a mono-cyclic terpene.



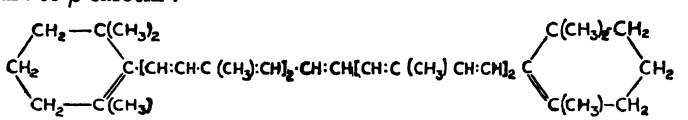
Camphor,
a bi-cyclic terpene.

Terpenes have been detected in over 250 plant species. They are widely distributed among 50 families, such as the Coniferae, Rutaceae, Myrtaceae, Labiatae, and Umbelliferae. Certain terpenes may be confined to a single genus or species, while other individual terpenes occur in widely different genera. Moreover, a single plant may produce more than one terpene. These compounds have been found in different parts of the shoot system, and are frequently produced by specialized secretory glands. As examples of the distribution of terpenes, we note that the secretory cells of the rind of the lemon and of certain other citrus fruits contain citronellol, geranial, and limonene ; that the leaves of the peppermint contain menthol ; that pinene is represented in the resin exuded from the stems of conifers ; and that camphor is found in the wood of the camphor tree.

The term resin denotes certain acidic substances which occur in plants (*e.g.*, pines and other conifers, the buds of horse chestnut and certain species of poplar, etc.), either as amorphous vitreous solids, or in solution in essential oils, *i.e.*, as balsams. Chemically, the resins form a mixed group. Some resins are phenolic derivatives ; others are oxidation products of terpenes. For example, after the distillation of the balsam, turpentine, there is left a residue, colophonium, from which a well-defined resin acid, abietic acid, $C_{20}H_{30}O_2$, has been isolated. The carbon skeleton of this acid is entirely composed of isoprene units.

(ii) *Carotinoids* (*or, alternatively, carotenoids*). Isoprene is represented in the molecular structure of the carotinoids. The carotinoids are yellow, orange, red, or brown pigments, which are insoluble in water,[1] but dissolve in ether, chloroform, and other solvents which dissolve fats. At least two carotinoids occur in every chloroplast, while non-green chromoplasts may contain several of these pigments. Some of these pigments (*e.g.*, carotin and lycopin) are hydrocarbons, others (*e.g.*, xanthophyll) contain alcholic groups, and a few (*e.g.*, crocetin) are carboxylic acids.

It appears that carotin, $C_{40}H_{56}$, which is an invariable component of all chloroplasts, exists in several isomeric forms, which differ only slightly in their molecular structure. Karrer has assigned the following structural formula to β-carotin :—



[1] *Cf.* the anthoxanthic and anthocyan pigments (section F).

It will be noticed that it is an unsaturated hydrocarbon with a long straight chain which terminates in homocyclic groups, viz., those of β-ionone.

Karrer assigned an open chain structure to lycopin, $C_{40}H_{56}$, which is found in the plastids of red tomatoes and of red pepper :—

$$\begin{matrix} CH\,(CH{:}C\,(CH_3){\cdot}CH{:}CH)_2{\cdot}CH{:}C\,(CH_3){\cdot}(CH_2)_2{\cdot}CH{:}C\,(CH_3)_2 \\ \| \\ CH\,(CH{\cdot}C\,(CH_3){\cdot}CH{:}CH)_2{\cdot}CH{:}C\,(CH_3){\cdot}(CH_2)_2{\cdot}CH{:}C\,(CH_3)_2 \end{matrix}$$

Carotinoid pigments may contain two or more hydroxyl groups. Thus the molecular formula of xanthophyll, which occurs with carotin in chloroplasts, is $C_{40}H_{56}O_2$, and a number of different forms have been recognized. Fucoxanthin, $C_{40}H_{56}O_6$, is the carotinoid which gives the brown colour to the Phaeophyceae.

Carotinoids have been intensively studied since it was discovered that they may give rise in the animal body to vitamin A. This vitamin is of special interest at the time of writing (January 1940), since, among other functions, by promoting the formation of the retinal substance, visual purple, it makes less acute the disability known as night blindness. Tswett's chromatographic method has been much used in recent experiments. When a solution containing carotinoids is caused to percolate through a column of dry adsorbent substance (*e.g.*, chalk, kaolin, powdered cane sugar), the individual carotinoids (carotins and xanthophylls) are differentially adsorbed. The different carotinoids are separated from one another by cutting up the pigmented stratified column and analysing the pigments adsorbed in the different regions thereof. It is now established that only such carotinoids as contain at least half a β-carotin molecule can give rise to vitamin A. Carrots are a rich source of provitamin A ; tomatoes contain little or none.

(iii) *Phytol.* Phytyl alcohol or phytol, $C_{20}H_{39}OH$, which exists in ester combination in chlorophyll (p. 442) and vitamin K (p. 12), has now been synthesized. Fischer and Löwenberg have assigned the following formula to phytol :—

$$(CH_3)_2CH.CH_2.CH_2.CH_2.CH(CH_3).CH_2.CH_2.CH_2.CH(CH_3).CH_2.$$
$$CH_2.CH_2.C(CH_3){:}CH.CH_2OH.$$

This structural formula suggests that similar metabolic processes (cleavage, reduction, condensation, etc.) govern the formation of phytol, and that of the yellow and orange plastid pigments.

(iv) *Sterols.* The protoplasm of all living cells contains at least one representative of a group of substances termed sterols. Sex hormones, and other substances which determine the behaviour of animal cells, belong to this group (see Needham, *206*). Their functions in plants have not yet been elucidated. The principal animal sterol is called cholesterol, $C_{27}H_{45}OH$. This is a cyclic compound composed of fused reduced benzene rings with side chains. It contains an unsaturated linkage and is a monohydric alcohol. Although sterols are not entirely composed of isoprene units, there are strong indications that there is some similarity between the anabolic events by which sterols are produced, and those which yield the carotinoids. Doubtless the phytosterols, for example sitosterol, $C_{29}H_{49}OH$, and stigmatosterol, $C_{29}H_{47}OH$, are built on a similar structural plan.

The sterols occur in association with true fats. They are soluble in fat solvents, but, being alcoholic, are not saponified by caustic alkalies (p. 423). They constitute what is called the unsaponifiable residue. As

they are quite insoluble in aqueous solutions, they may be separated from soaps by filtration.

(v) *Sapogenins.* Certain glycosides called saponins are built up from sugars and substances called sapogenins, which appear to be constructed from isoprene units. Sapogenins have been classified among the triterpenes.

B. Aldehydes and Ketones

We have already noted that aldehydes and ketones are represented in plants in the terpene group of essential oils (footnote, p. 415). Cinnamic aldehyde, which is the chief constituent of oil of cinnamon, and vanillic aldehyde (vanillin), which is responsible for the scent of vanilla pods, provide additional examples of aldehyde components of essential oils.

Several aldehydes, for example benzaldehyde, and the simplest ketone, acetone, occur in plants, combined as glycosides (section E). It has been suggested that vanillin, $C_6H_3(OH)(OCH_3)(CHO)$ [1 : 2 : 4], or coniferyl aldehyde,

$$C_6H_3(OH)(OCH_3)(CH : CH.CHO)\ [1 : 2 : 4],$$

may enter into the composition of lignin.

The oxidation and reduction of aldehydes and ketones play an important part in intermediate metabolism. Aldehydes, R.CHO, are produced by the oxidation of primary alcohols, $R.CH_2OH$. Denoting the oxidizing agent by A, we may write :

$$2R.CH_2OH + A \longrightarrow 2R.CHO + AH_2.$$

This type of change is called oxidation by dehydrogenation : the alcohol upon losing hydrogen, is oxidized, and the oxidizing agent, upon accepting hydrogen, is reduced. By such oxidations formaldehyde, HCHO, would be produced from methyl alcohol; acetaldehyde, $CH_3.CHO$, from ethyl alcohol; benzaldehyde, $C_6H_5.CHO$, from benzyl alcohol; cinnamic aldehyde, $C_6H_5.CH : CH.CHO$, from cinnamic alcohol; and glycollic aldehyde, $CH_2OH.CHO$, and glyoxal, CHO.CHO, from ethylene glycol.

These oxidations can be reversed, since aldehydes are easily reduced to the corresponding alcohols. This type of reduction results from the acceptance by the aldehyde of labile hydrogen from a so-called hydrogen donator. In the following equation

$$R.CHO + AH_2 \longrightarrow R.CH_2OH + A$$

AH_2 is the hydrogen donator that acts as the reducing agent. It is itself oxidized by dehydrogenation. R.CHO is the hydrogen acceptor and is reduced. Furthermore, aldehydes are readily oxidized and give rise to organic acids,

$$2R.CHO + O_2 \longrightarrow 2R.COOH.$$

This equation suggests that oxidations may be brought about by the direct addition of oxygen (additive oxidation). Some authorities consider that even in this transformation, oxidation takes place by the dehydrogenation of a hydrated aldehyde, $R.CH(OH)_2$, and that oxygen acts as a hydrogen acceptor. Upon oxidation, formaldehyde gives formic acid, H.COOH; acetaldehyde gives acetic acid, $CH_3.COOH$; benzaldehyde gives benzoic acid, $C_6H_5.COOH$; cinnamic aldehyde gives cinnamic acid, $C_6H_5.CH : CH.COOH$; glycollic aldehyde successively gives glycollic acid, $CH_2OH.COOH$, glyoxylic acid, CHO.COOH, and oxalic acid, COOH.COOH; and glyoxal gives glyoxylic and oxalic acids.

The Cannizzaro reaction provides the type reaction, of a hydrolytic oxidation reduction known as a dismutation, in which one of a pair of

molecules of a single aldehyde is reduced to the corresponding alcohol, while the other is oxidized to the corresponding acid.

$$R.CHO + H_2O + R.CHO = R.CH_2OH + R.COOH.$$

In recent years the use of the term dismutation has been extended to include oxidoreductions in which different aldehydes, or an aldehyde and a ketonic substance, participate and give rise, in each reaction, to equimolecular amounts of alcohol and acid.

Ketones ($R_1.CO.R_2$) are the first products to arise when secondary alcohols ($R_1.CHOH.R_2$) are oxidized.

$$2R_1.CHOH.R_2 + O_2 \longrightarrow 2R_1.CO.R_2 + 2H_2O.$$

The mechanism of this oxidation is the same as that of the oxidation of aldehydes. Moreover, ketones, like aldehydes, are easily reduced to the corresponding alcohol. Acetone, $CH_3.CO.CH_3$, the simplest ketone, has not been detected in the free state in plants living under normal conditions, but occurs in the combined state in certain cyanogenetic glucosides. The corresponding secondary alcohol is isopropyl alcohol, $CH_3.CHOH.CH_3$.

Whereas in straight-chain aldehydic compounds, the aldehydic group is necessarily terminal, in straight-chain ketonic compounds the ketonic group is intercalated (*cf.* the formulæ for glucose and fructose in the next section). Hence ketones, unlike aldehydes, may give rise to two or more organic acids when they are oxidized.

The molecules of aldehydes and ketones contain a carbonyl group. In consequence these substances show the properties of unsaturated compounds. For instance, they readily form additive compounds with (i) sodium bisulphite and with (ii) hydrogen cyanide.

(i) $R.C\begin{matrix}H\\ \diagup\\ \diagdown\\ O\end{matrix} + NaHSO_3 \rightarrow R.CH(OH).SO_3Na$ (aldehyde bisulphite).

$\begin{matrix}R\\ \diagup\\ \diagdown\\ R\end{matrix}C = O + NaHSO_3 \rightarrow \begin{matrix}R\\ \diagup\\ \diagdown\\ R\end{matrix}C(OH).SO_3Na$ (ketone bisulphite).

(ii) $R.CHO + HCN \rightarrow R.CH(OH).CN$ (aldehydecyanhydrin)[1]

$\begin{matrix}R\\ \diagup\\ \diagdown\\ R\end{matrix}C = O + HCN \rightarrow \begin{matrix}R\\ \diagup\\ \diagdown\\ R\end{matrix}C(OH).CN$ (ketonecyanhydrin).

In addition the presence of the carbonyl group renders labile and reactive the hydrogen atoms attached to the α-carbon atom. Consequently aldehydes and ketones can undergo condensations. The aldol condensation, which occurs with great readiness, depends upon (*a*) the unsaturation of the carbonyl group, and (*b*) the lability of the α-hydrogen atoms. From acetaldehyde, ordinary aldol is produced :

$$CH_3.CH = O + H.CH_2.CHO \longrightarrow CH_3.CH(OH).CH_2.CHO$$

ordinary aldol.

Upon dehydration this product is converted into crotonic aldehyde, $CH_3.CH : CH.CHO$. The labile hydrogen atoms are confined to the α-carbon atoms in saturated aldehydes, but in αβ-unsaturated aldehydes, such as crotonic aldehyde, the reactivity can be transmitted to the γ-carbon

[1] The cyanhydrin of benzaldehyde, $C_6H_5.CH(OH).CN$, is of considerable interest to botanists (see p. 434). It is known as mandelonitrile, since it is converted by hydrolysis into mandelic acid, $C_6H_5.CH(OH).COOH$.

atom. Accordingly acetaldehyde and crotonic aldehyde can condense thus :

$$CH_3.CHO + H_2|CH.CH:CH.CHO \longrightarrow CH_3.CH:CH.CH:CH.CHO + H_2O.$$

Aldehydes, unlike ketones, show a great tendency to polymerize, *i.e.*, the molecules possess the power of combining with one another to yield a substance with the same empirical formula, but with a higher molecular weight. The formation of aldol from acetaldehyde represents one form of polymerization. Formaldehyde may give rise to several polymers ; for example, a mixture of sugars called formose is produced by the action of dilute alkalies on solutions of formaldehyde :

$$6\,HCHO \longrightarrow C_6H_{12}O_6.$$

On the basis of this reaction, Baeyer advanced the formaldehyde hypothesis of photosynthesis, which is now out of favour (p. 235).

Certain important compounds contain both ketonic and aldehydic groupings. They possess most of the general properties mentioned above. Glyoxal (CHO.CHO) is the type compound, and pyruvic aldehyde or methyl-glyoxal, $CH_3.CO.CHO$, is a derivative.

C. Organic Acids containing Carbon, Hydrogen, and Oxygen, and their Derivatives

Salt and ester formation ; reversible reactions. Organic acids (R.COOH) undergo electrolytic dissociation in water, and furnish hydrogen ions, H^+, and organic anions, $R.COO^-$. The organic anion combines with metallic ions to form molecules of salts, such as R.COONa, $(R.COONa)_2$, R.(COOH)(COONa), and $R.(COO)_2Ca$. Such salts are widely distributed in plants. When organic acids combine with alcohols, water is eliminated and organic salts called esters are formed :

$$\underset{\text{organic acid.}}{R_1.COOH} + \underset{\text{alcohol.}}{R_2OH} \longrightarrow \underset{\text{ester.}}{R_1.COOR_2} + \underset{\text{water.}}{H_2O}.$$

This type of reaction, viz., the condensation of two organic molecules with the elimination of one molecule of water, takes place in all living cells. The reverse process, viz., cleavage with the addition of a molecule of water, is called a hydrolysis. A condensation which leads to the formation of an ester as represented in the above equation is described as an esterification. The alcohol and acid are regenerated by the hydrolysis of the ester :

$$R_1.COOR_2 + H_2O \longrightarrow R_1.COOH + R_2OH.$$

The fact that esterification and hydrolysis are reversible reactions may be symbolically represented by combining the above equations thus :

$$R_1COOH + R_2OH \underset{\text{hydrolysis } (v_2)}{\overset{\text{esterification } (v_1)}{\rightleftharpoons}} R_1COOR_2 + H_2O.$$

This equation implies that whether the reaction is started by mixing acid and alcohol, or by mixing ester and water, the final result will be an equilibrium mixture of acid, alcohol, ester, and water. Let us suppose that the velocity of esterification at any time t during the course of the reaction is v_1 and that simultaneously hydrolysis is occurring at a velocity of v_2. Until equilibrium is reached v_1 will differ from v_2. Now the law of mass-

action states that the rate of a chemical reaction is proportional to the concentration of the reacting substances. Hence, at any time

$$v_1 = k_1 [R_1.COOH] [R_2OH]$$
$$v_2 = k_2 [R_1.COOR_2] [H_2O]$$

where the bracketed formulæ represent the concentrations of acid, alcohol, ester, and water, at the time t, and k_1, k_2 are the velocity constants of the esterification and hydrolysis, respectively. At equilibrium the composition of the mixture of the four substances will remain constant. This equilibrium is dynamic, for esterification and hydrolysis are still proceeding, but at equal rates. Since $v_1 = v_2$ at equilibrium,

$$k_1[R_1.COOH] [R_2OH] = k_2[R_1.COOR_2] [H_2O]$$

or,

$$\frac{[R_1.COOH] [R_2OH]}{[R_1.COOR_2] [H_2O]} = \frac{k_2}{k_1} \quad \text{K (the equilibrium constant).}$$

The value of K is independent of the initial composition of the reaction mixture. Hence it follows that when dilute solutions are used, the percentage of acid and alcohol present at equilibrium will be relatively high, and the percentage of ester relatively low, *i.e.*, hydrolysis will be favoured. Conversely, if concentrated solutions of acid and alcohol are used, esterification will be favoured.

The time of reaching equilibrium in these reversible systems may be shortened by the addition of mineral acids to the reaction mixture. These acids take no part in the reaction, and are unchanged at the end of the reaction, *i.e.*, they act as catalysts. Since the catalyst enhances v_1 and v_2 equally, the equilibrium constant is not altered.

Equilibrium will be disturbed in both catalyzed and uncatalyzed reversible reactions if one of the products of the reaction is removed after equilibrium has been attained. Such removal could be effected in the system now under discussion either by the distillation of the ester or the alcohol, or by the formation of the sodium salt of the acid upon adding alkali to the equilibrium mixture. The production of the substance which is removed would continue until dynamic equilibrium is once again reached or until one of the components of the reaction has completely disappeared. This principle will be illustrated below, when saponification is considered. It is of importance in the theoretical treatment of problems of the chemical dynamics of living cells in which continuous reactions occur in certain directions as a result of the incessant production and removal of substances. Equilibrated states can only be attained when removal ceases.

Monobasic aliphatic acids and fatty acids, and their esters. Fats. (i) *Fatty acids.* Acids belonging to this series possess an open-chain structure terminating with a carboxylic group. They may contain one or more unsaturated linkages. In the so-called higher fatty acids the length of the chain is considerable. In the lower members it is short. Among the lower members, acids possessing branched chains may be produced during the course of plant metabolism. Neither those with an odd number of carbon atoms, nor those with a branched chain are found among the higher members which are represented in plant fats. Saturated and unsaturated acids containing eighteen carbon atoms occur frequently in plant fats.

The lower members of the fatty acids are soluble in water. The solubility decreases as we ascend each homologous series. The higher members (say

the C_{16} and C_{18} acids) are insoluble, and possess an oily or, if solid, a waxy consistency.

Classification of Fatty Acids

Saturated fatty acids $C_nH_{2n}O_2$. Formic acid, H.COOH; acetic acid, $CH_3.COOH$; normal butyric acid, $C_3H_7.COOH$; iso-valeric acid, $(CH_3)_2CH.CH_2.COOH$; caproic acid, $CH_3.(CH_2)_4.COOH$; caprylic acid, $CH_3.(CH_2)_6.COOH$; palmitic acid, $CH_3.(CH_2)_{14}.COOH$; stearic acid, $CH_3.(CH_2)_{16}.COOH$; and carnaubic acid, $CH_3.(CH_2)_{22}.COOH$.

Unsaturated fatty acids, $C_nH_{2n-x}O_2$ (where $x = 2, 4, 6$, or 8). Oleic acid, $C_{18}H_{34}O_2$, or $CH_3.(CH_2)_7.CH:CH.(CH_2)_7.COOH$; linolic acid, $C_{18}H_{32}O_2$; linolenic acid, $C_{18}H_{30}O_2$.

Unsaturated hydroxy fatty acids, *e.g.*, Ricinoleic acid, $C_{17}H_{32}(OH)COOH$.

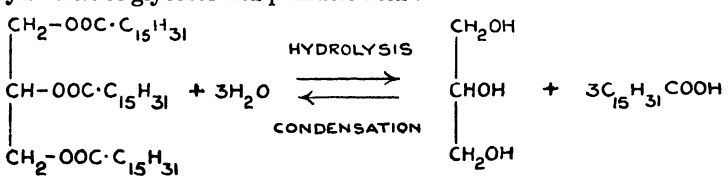
The higher fatty acids may accumulate in plant cells when fats are hydrolyzed. Some of the others occur sporadically in the free state. Formic acid may, by microchemical tests, be readily detected in the stinging hairs of the nettle. Acetic acid probably plays an important part in plant metabolism, since it is an oxidation product of acetaldehyde.

(ii) *Volatile esters.* Esters of fatty acids are widely distributed in nature. Certain volatile esters are members of a group of the substances known as essential oils (p. 415). They must be distinguished from fatty oils (see below). The fragrance of certain fruits results from the production of volatile esters (p. 212).

(iii) *Waxes.* Esters of higher alcohols with higher aliphatic acids have been found among the components of the wax-like coverings of certain plant members (see also p. 413). For example, carnaubyl carnaubate, $C_{23}H_{47}.COOC_{24}H_{49}$, an ester of carnaubic acid, $C_{23}H_{47}.COOH$, with carnaubyl alcohol, $C_{24}H_{49}OH$, occurs in the wax on the leaves of the Brazilian palm.

(iv) *Fats.* Fats are esters which are produced by the condensation of three molecules of the same or of different fatty acids with glycerol. Plant fats are liquid at ordinary temperatures, and are, in consequence, often called fatty oils. It is an important fact to note that only those fatty acids which contain an even number of carbon atoms (*e.g.*, acetic, butyric, caproic, palmitic, stearic, oleic, linolic, linolenic, and ricinoleic) occur in fats. Moreover, acids with branched chains have not yet been discovered in fats.

Fats are present in all living cells, but are most abundantly found in the storage tissue of the so-called oily seeds. Typical of the names given to fatty oils are palmitin (which occurs in palm kernels), olein (which occurs in the fruit of the olive), and butyrin (which occurs with palmitin and olein in coconut endosperm). When these fats are hydrolyzed they yield the corresponding fatty acids and glycerol. Hydrolysis may be catalyzed by acids or alkalies, or by the enzyme lipase. For instance, glycerol and palmitic acid are produced by the hydrolysis of palmitin. This fat is probably formed in ripening palm kernels by the condensation with dehydration of glycerol and palmitic acid :



Palmitin, or glycerol tripalmitate. Glycerol. Palmitic acid.

This reversible reaction provides us with an example of the generalized system discussed on page 420.

Hydrolysis carried out in alkaline solution is called saponification, because the alkaline salts of some of the fatty acids (*e.g.*, sodium palmitate), are used as soaps.

$$(C_{15}H_{31}COO)_3.C_3H_5 + 3NaOH \longrightarrow 3C_{15}H_{31}COONa + C_3H_5(OH)_3.$$

If excess of alkali is used, the fat will be completely saponified, since, by combining with the alkali, the fatty acids set free will be immediately removed from the reacting system.

Fats are insoluble in water, but dissolve in ether, petroleum ether, chloroform, and certain other solvents.[1]

Substituted monobasic aliphatic acids. Many monohydroxy monobasic aliphatic acids play an important part in plant metabolism. The carbon atom adjoining the carboxylic group in a monobasic acid is known as the α-carbon atom, the next as the β-carbon atom, and so forth. Thus glycollic acid, $CH_2OH.COOH$, is α-hydroxy-acetic acid; and lactic acid, $CH_3.CHOH.COOH$ is α-hydroxy-propionic acid. The α-carbon atom in lactic acid is asymmetric (see p. 424); consequently this acid is optically active. The dextrorotatory form accumulates in the muscle tissue of animals during exercise. Some of the derivatives of β-hydroxy-propionic acid, $CH_2OH.CH_2.COOH$, occur in nature.

The α-ketonic acid, pyruvic acid, $CH_3.CO.COOH$, and its enol, $CH_2:C(OH).COOH$, play an important part in plant metabolism. *In vivo* pyruvic acid is probably derived from phosphoglyceric acid (p. 55). *In vitro* it can be obtained by the oxidation of methylglyoxal, and on reduction it yields lactic acid. Carbon dioxide is eliminated (decarboxylation) and aldehydes are formed when many α-ketonic acids are acted on by the enzyme, carboxylase. For instance, acetaldehyde and carbon dioxide are produced as a result of the decarboxylation of pyruvic acid.

Polyhydroxy-monobasic acids are produced by the partial oxidation of monosaccharides. For example, gluconic, galactonic, and mannonic acids, might all be described as $\alpha\beta\gamma\delta\epsilon$-pentahydroxy-caproic acids,

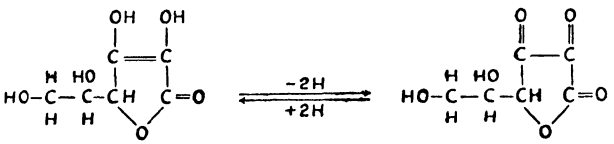
$$CH_2OH.(CHOH)_4.COOH.$$

Citrus fruits and many other fresh plant tissues produce by their metabolism a substance which, when fed to animals in minute amounts, arms them against the affliction known as scurvy. For many years this substance has been described by the name vitamin C.[2] Its structure has been established, and it was the first vitamin to be synthesized by chemists. Because of its special curative properties the pure substance is called

[1] In addition sterols, lecithins, chlorophyll, the carotinoids, waxes, and other substances, are soluble in the above-named fat solvents. It should be noted that proteins and carbohydrates are insoluble in these solvents.

[2] Among the other products of plant metabolism that may themselves act as vitamins in animal nutrition, or give rise in the animal body to vitamins, are the carotinoids (p. 416), which are considered to be the precursors of vitamin A; aneurin or thiamin (see p. 445), which is supposed to be identical with vitamin B_1; flavins (p. 444), nicotinic acid derivatives (p. 444), pyridoxin (p. 440), and pantothenic acid (p. 449), which may be among the products known as the vitamin B complex; certain sterols (p. 417), to which chemical group vitamin D unquestionably belongs, and to which group vitamin E may have chemical affinities; biotin (p. 445), which has the physiological properties of vitamin H; and the substituted naphthoquinone that gives to chloroplasts (p. 12) the powers of vitamin K. For further information see Rosenberg (*252*).

ascorbic acid ($C_6H_8O_6$). Between *pH* values of 1·0 and 4·5 ascorbic acid may be dehydrogenated by mild oxidizing agents, such as iodine or 2, 6-dichlorophenol-indophenol (a blue redox indicator) to yield dehydroascorbic acid ($C_6H_6O_6$). This oxidation may be reversed by adding a suitable reducing agent, *e.g.*, hydrogen sulphide. With mild oxidizing agents at *pH* values greater than 4·2 or with strong oxidizing agents at any *pH* value, irreversible oxidation occurs. Products such as oxalic acid and threonic acid are formed. All these facts of chemistry may bear on metabolic problems (p. 41). The affinity borne to hexose sugar of the two forms should be noted. It is not known which of the two is first produced in metabolism.



Ascorbic acid. Dehydroascorbic acid.

Dibasic and tribasic aliphatic acids.[1] A list is given below of the di- and tricarboxylic acids mentioned in chap. XIV and elsewhere in this book.

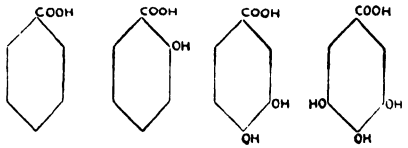
Oxalic acid	HOOC.COOH
Malonic acid	HOOC.CH_2.COOH
Succinic acid	HOOC.CH_2.CH_2.COOH
Malic acid	HOOC.CH_2.$\overset{*}{C}$H(OH).COOH
Oxalacetic acid	HOOC.CH_2.CO.COOH
Tartaric acid	HOOC.$\overset{*}{C}$H(OH).$\overset{*}{C}$H(OH).COOH
Glutaric acid	HOOC.CH_2.CH_2.CH_2.COOH
α-Ketoglutaric acid	HOOC.CH_2.CH_2.CO.COOH
Tricarballylic acid	HOOC.CH_2.CH(COOH).CH_2.COOH
Cisaconitic acid	HOOC.CH=C(COOH).CH_2.COOH
Citric acid	HOOC.CH_2.$\overset{*}{C}$(OH)(COOH).CH_2.COOH
Isocitric acid	HOOC.$\overset{*}{C}$H(OH).$\overset{*}{C}$H(COOH).CH_2.COOH

Some of the polyhydroxy dibasic acids are related to the sugars. Of the ten stereoisomeric acids possessing the formula HOOC.($\overset{*}{C}$HOH)$_3$.COOH, we mention saccharic acid, obtained by the oxidation of glucose, and mucic acid by that of galactose.

Aromatic acids. Aromatic acids occur in the free state, and also combined as esters and glycosides. Tannins, which are found in colloidal solution in the sap of many plant cells, are produced by the condensation of certain phenolic acids.

We shall consider only a few examples of a numerous group. Benzoic acid, C_6H_5.COOH, does not occur in the free state but may be obtained by heating a plant resin called gum benzoin. Salicylic acid, C_6H_4(OH).COOH, is found as the methyl ester in *Gaultheria procumbens*. Protocatechuic acid, $C_6H_3(OH)_2$.COOH, has been found in the scale leaves of the onion bulb. Gallic acid, $C_6H_2(OH)_3$.COOH, occurs in the leaves of Thea (tea).

[1] We shall denote asymmetric carbon atoms by means of asterisks. This has already been done for lactic acid. Fumaric acid, HOOC . CH=CH . COOH, should also be added to the list.



Benzoic acid. Salicylic acid. Protocatechuic acid. Gallic acid.

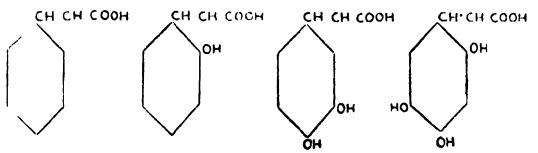
It should be noted that protocatechuic and gallic acids are derivatives of catechol and pyrogallol respectively. These acids are the units from which the complex tannins[1] are built. For example, two molecules of gallic acid condense to give what is called a didepside :

$$C_6H_2(OH)_3.COOH + OH.C_6H_2(OH)_2.COOH \longrightarrow C_6H_2(OH)_3.CO\text{-}O\text{-}C_6H_2(OH)_2.COOH + H_2O.$$

Didepside of gallic acid.

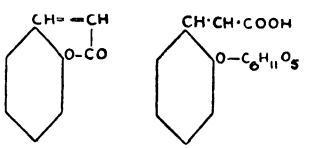
Further condensation leads to the formation of tridepsides, and so forth. Sometimes the depsides unite with glucose to give glucosides : for example gallotannic acid, the tannin contained in oak galls, is a compound in which didepside residues of gallic acid are combined with the five free hydroxyl groups of ordinary glucose. It may therefore be called pentadigalloyl glucose.

Hydroxy derivatives of cinnamic acid, $C_6H_5.CH : CH.COOH$, form an interesting series of aromatic acids which contain an unsaturated linkage in the side chain. Ortho-coumaric acid, caffeic acid, and aesculetic acid, are



Cinnamic acid. *o*-Coumaric acid. Caffeic acid. Aesculetic acid.

found in glycosidal union. In *o*-coumaric acid and in aesculetic acid internal condensation with the formation of the lactones *o*-coumarin and aesculetin readily takes place owing to the proximity of the carboxylic and hydroxyl groups. *o*-Coumarin is responsible for the fragrant odour of hay



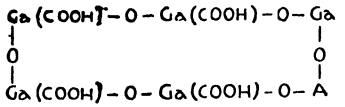
o-Coumarin. *o*-Coumaric acid glucoside.

containing sweet vernal grass, and of the tonka bean used in perfumeries. In the late spring, the scent of *o*-coumarin is strong in woods in which sweet woodruff is withering. The healthy living cells of these plants contain a

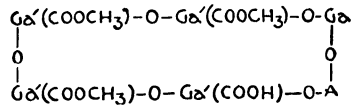
[1] The catechol tannins are derived from protocatechuic acid, and the pyrogallol tannins from gallic acid. Aqueous solutions of catechol tannins are coloured green and of pyrogallol tannins dark blue by ferric chloride.

non-odorous glucoside of coumaric acid, which is hydrolyzed during late senescence. *o*-Coumaric acid is thus set free, and undergoes internal condensation with the elimination of water to give *o*-coumarin.

Complex acidic substances. (i) *Pectic substances.* The pectic substances of plants have been subjected to much investigation because they are of commercial importance in the jam-making industries. Protopectin (which used to be called pectose) is the name given to the pectic component of cell walls. This substance may possibly exist in loose chemical combination with cellulose. It can be separated from cell walls as commercial pectin or pectinogen. Pectin is soluble in water, and occurs in the cell sap of many plant organs (*e.g.*, succulent fruits). Pectin is either a tri- or a tetra-methyl-ester of methylated pectic acid. In the structural formulæ given below for this acid, and for pectin, residues of galactose, galacturonic acid, methylated galacturonic acid, and arabinose, are represented by Ga, Ga(COOH), Ga′(COOH), and A, respectively. Pectic acid may be described as galacto-arabino-tetra-galacturonic acid. The sugar and sugar-acid residues are connected by glycosidal linkages.



Pectic acid
(galacto-arabino-tetra-galacturonic acid).



Pectin.

Calcium, magnesium, iron, and other metallic elements, may occur in combination with the carboxylic groups in pectin or pectic acid. It has long been known that calcium pectate is a component of the middle lamella of cell walls.

(ii) *Gums.* The gums found in plants are mixtures of different compounds. They always contain pentosans. For instance, araban is a component of gum arabic, and xylan occurs in wound gum from wood. Gums may also contain hexosans. They are included in this section because they always contain organic acids. The composition of these acids is unknown.

(iii) *Cutin and suberin.* Both the cutin of plant cuticle and the suberin of cork are mixtures of several compounds, viz., condensation and oxidation products of certain unsaturated fatty acids, and esters and soaps of these acidic products. The following acids have been recognized among the products obtained by hydrolyzing suberin with alcoholic soda : phellonic acid or α-hydroxy-behenic acid, $CH_3.(CH_2)_{19}.CHOH.COOH$; phloionic acid, $C_{18}H_{34}O_6$, a dibasic acid ; phloionolic acid or trihydroxy-stearic acid, $C_{18}H_{36}O_5$; and corticinic and suberolic acids, whose composition is not yet determined.

D. Carbohydrates

Most of the naturally occurring carbohydrates may be represented by the general formula, $C_x(H_2O)_y$. Rhamnose ($C_6H_{12}O_5$), a methyl pentose· is, however, a well-known exception. Carbohydrates can be classified according to their relative complexity as (*a*) monosaccharides ; (*b*) di-, tri-, and tetra-saccharides ; and (*c*) polysaccharides. The members of groups (*a*) and (*b*) are called sugars. They dissolve in water, giving crystalloid solutions with a sweet taste. An essential part of the structure of a sugar is an aldehydic or a ketonic group, in association with one or more alcoholic groupings.

Monosaccharides. The general formula of most of the naturally occurring monosaccharides is $C_x(H_2O)_x$. By classifying monosaccharides according to the number of carbon atoms in the molecule, we may recognize sub-groups of sugars termed dioses, trioses, tetroses, pentoses, and hexoses. Other sub-groups in which the value of x is greater than 6 are known.

Monosaccharides containing aldehyde groupings are called aldoses, and those containing ketonic groupings are called ketoses.

(i) *Dioses, trioses, and tetroses.* Glycollic aldehyde, $C_2H_4O_2$ or $CH_2OH.CHO$, is the simplest monosaccharide. Its properties will evidently be those of a primary alcohol and of an aldehyde. Reduction gives glycol, $CH_2OH.CH_2OH$, oxidation proceeds *vid* glycollic acid, $CH_2OH.COOH$, to oxalic acid, $COOH.COOH$. Dihydroxy acetone, $CH_2OH.CO.CH_2OH$, is a ketotriose which yields glycerol on reduction. Glycerol is also formed by the reduction of an aldotriose, viz., glyceric aldehyde, $CH_2OH.\overset{*}{C}HOH.CHO$. This compound contains an asymmetric carbon atom, and *d*-, *l*-, and racemic, forms, are known. On reduction, asymmetry is destroyed ; consequently glycerol is not optically active. Triosephosphates play an exceedingly important part in plant metabolism.

Isomers become increasingly numerous in the more complex monosaccharides. At least six tetroses, $C_4H_8O_4$, exist. There are two optically active ketotetroses, $CH_2OH.CO.\overset{*}{C}HOH.CH_2OH$, and four optically active aldotetroses, $CH_2OH.\overset{*}{C}HOH.\overset{*}{C}HOH.CHO$. It should be noted that in the aldotetroses the two asymmetric carbon atoms act independently in producing optical isomers. An aldotetrose is converted, by oxidation, into one or more of the tartaric acids, $COOH.\overset{*}{C}HOH.\overset{*}{C}HOH.COOH$.

(ii) *Pentoses.* The pentose sugars, $C_5H_{10}O_5$, comprise aldoses and ketoses, but only the former are of interest to botanists. Four pairs of optical isomers can exist in the aldopentoses,

$$CH_2OH.\overset{*}{C}HOH.\overset{*}{C}HOH.\overset{*}{C}HOH.CHO.$$

These have been named *d*- and *l*-xylose, *d*- and *l*-arabinose, *d*- and *l*-ribose, and *d*- and *l*-lyxose. Of these, only *l*-arabinose, *d*-xylose, and *d*-ribose, are represented in plants.[1] It is doubtful whether they occur in the free state, but *d*-xylose and *l*-arabinose are widely distributed as condensation products in the polysaccharides called pentosans, and in certain glycosides. The active form of *d*-ribose, known as *d*-ribofuranose

$$(CH_2OH.\overset{\overset{\diagup\!\!\diagdown}{O}}{\underset{\diagdown\!\!\diagup}{CH}}.(CHOH)_2.CHOH)$$

occurs in yeast nucleic acid and in certain coenzymes (p. 444). Its derivative, desoxyribofuranose ($CH_2OH.\overset{\overset{\diagup\!\!\diagdown}{O}}{\underset{\diagdown\!\!\diagup}{CH}}.CHOH.CH_2.CHOH$) is found in thymus nucleic acid.

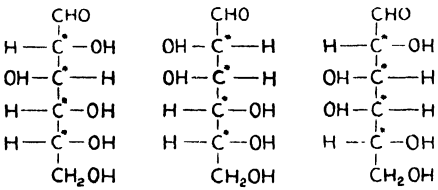
(iii) *Hexoses.* Hexose sugars ($C_6H_{12}O_6$) are present in all living cells. They constitute the reducing sugars in plants, and form osazones (see Onslow, *216*). Eight pairs of optically active aldohexoses,

$$CH_2OH.\overset{*}{C}HOH.\overset{*}{C}HOH.\overset{*}{C}HOH.\overset{*}{C}HOH.CHO,$$

are known to chemists, viz., *d*- and *l*- forms of glucose, mannose, galactose, idose, gulose, talose, allose, and altrose. Our task is simplified since of

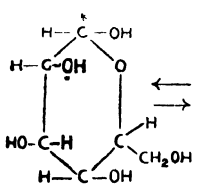
[1] Possible variations in the molecular structure of a given pentose are indicated on p. 429.

these isomers only *d*-glucose, *d*-mannose, and *d*-galactose are represented in plants. The formulæ of the optical isomers, *l*-glucose, *l*-mannose, and *l*-galactose, would be mirror images of the formulæ drawn below. Only *d*-glucose has been found in the free state.

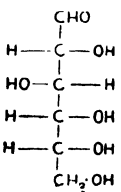


d-Glucose. *d*-Mannose. *d*-Galactose.

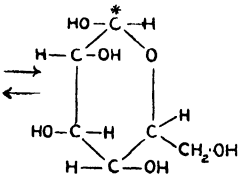
Recent work has shown that each hexose variety can exist in several distinct forms. Two forms, α- and β-, of *d*-glucose have been isolated. They are both dextrorotatory. The α-form has the stronger rotatory power. It has been observed that the optical rotation of freshly prepared aqueous solutions of the α-form steadily decreases, while that of solutions of the β-form steadily increases. Since in solutions of either form the same final equilibrium value is reached for the same initial amount of sugar, it has been concluded that glucose, when dissolved in water, quickly becomes an equilibrated mixture of the α- and β-forms. The changes in rotation comprise the phenomenon known as mutarotation. It is generally accepted that the existence of two forms of *d*-glucose results from the appearance of an additional asymmetric carbon atom (which is marked with an asterisk in the formulæ given below), when the sugar assumes a ring structure. It should be noticed that α- and β-glucose are stereoisomeric but not optically isomeric substances.



α-Glucose. (Amylene-oxide or pyranose form.)



(Intermediate phase during mutarotation.)

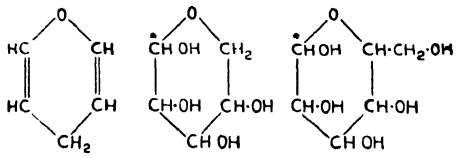


β-Glucose. (Amylene-oxide or pyranose form.)

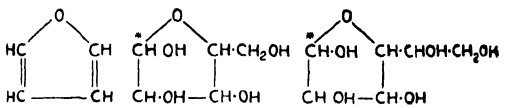
This ring structure may alternatively be termed the amylene-oxide ring (C—C—C—C—C—O), or the pyranose ring.[1] There is no definite evidence of the occurrence in nature of butylene-oxide (C—C—C—C—O) or furanose aldohexoses.[1] It was Haworth (*121*) who pointed out that amylene-oxide sugars may be regarded as derivatives of pyran, and butylene-oxide or γ-sugars as derivatives of furan. For pentose sugars, one may speak of pentopyranoses and pentofuranoses; and for hexose sugars, of hexo-

[1] The molecular structures of pyranose and furanose sugars and their derivatives are nowadays often depicted in perspective drawings (see fig. 2).

pyranoses and hexofuranoses. Naturally occurring glucose may be described as an equilibrated mixture of α- and β-forms of *d*-glucopyranose.

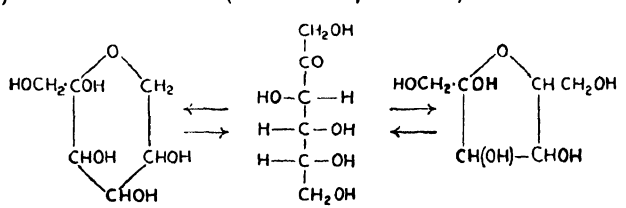


Pyran. Pyranose form of aldopentose. Pyranose form of aldohexose.
(Amylene-oxide or normal sugars.)



Furan. Furanose form of aldopentose. Furanose form of aldohexose.
(Butylene-oxide or active sugars.)

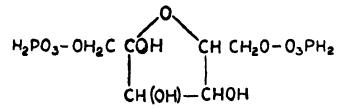
Of the many possible isomerides of ketohexoses possessing the formula, $CH_2OH.CO.\overset{*}{C}HOH.\overset{*}{C}HOH.\overset{*}{C}HOH.\overset{*}{C}H_2OH$, only fructose (or levulose) occurs in nature. It is probable that uncombined fructose is nearly always present in the cells of higher plants. It also occurs in combination with other sugars in cane sugar and raffinose ; and the polysaccharide, inulin, is a condensation product of fructose. Naturally occurring fructose is optically active and rotates the plane of polarized light to the left. It is, however, termed *d*-fructose, since it is stereochemically related to *d*-glucose. The amylene-oxide or pyranose formula has been assigned to uncombined stable fructose. Fructose exists in the butylene-oxide or furanose form when combined in cane sugar and in hexosephosphoric ester, and possibly also in inulin. This form is considerably more reactive than stable fructose, and it is probable that, preparatory to cellular oxidations and certain other metabolic events, protoplasmic systems convert fructopyranose (stable fructose) into fructofuranose (reactive or γ-fructose).



Fructopyranose (Normal fructose or the amylene-oxide form). Ketonic intermediate stage. Fructofuranose (γ-fructose or the butylene-oxide form).

Turning next to certain derivatives of the hexoses we note that several distinct hexosemonophosphates, $C_6H_{11}O_5(PO_4H_2)$, and a hexosediphosphate $C_6H_{10}O_4(PO_4H_2)_2$, have been isolated from the products of the alcoholic fermentation of hexoses by yeast in the presence of phosphates (see p. 52 and fig. 2). According to Morgan and Robison (*205*), the diphosphoric

ester of fructofuranose (*i.e.*, of active fructose) is produced, whichever sugar (glucose, fructose, or mannose) is fermented by yeast juice.



1 : 6-diphosphoric ester of γ-fructose.

The alcoholic grouping in sugars may, in addition to the ketonic or aldehydic groupings, undergo oxidation. Gluconic acid, $CH_2OH.(CHOH)_4.COOH$, arises when the aldehyde group in glucose is oxidized; by the oxidation of the secondary alcoholic group glucuronic acid, $COOH.(CHOH)_4.CHO$, is formed; and saccharic acid, $COOH.(CHOH)_4.COOH$ is produced by the oxidation of both of these groups. *d*-Galacturonic acid, $CHO.(CHOH)_4.COOH$, which is formed by the oxidation of galactose is a constituent of pectic acid. It has been suggested that *l*-arabinose is produced in the plant by the decarboxylation of *d*-galacturonic acid. The fact that *d*-galactose and *l*-arabinose are often represented together in complex polysaccharides has often been noted. There is a similar association between *d*-glucose and *d*-xylose. Possibly xylose is produced by the decarboxylation of glucuronic acid.

The keto-sugars, like ketones as a class, are cleaved by oxidation into two or more acids. Fructose gives tartaric and oxalic acids.

In water, or in weakly acid solutions, monosaccharides are very stable, but undergo interesting transformations in weakly alkaline solutions. For example, in N/20 calcium hydroxide, *d*-glucose, *d*-mannose, and *d*-fructose, are each converted into a mixture containing all three of these sugars. The structural similarity between the three sugars named should be noticed. It has been suggested that the transformation takes place *viâ* a common enol form, $CHOH:C(OH).(CHOH)_3.CH_2OH$. Galactose is never produced under these conditions.

In stronger alkalies (*e.g.*, 0·4N potassium hydroxide) oxygen is absorbed and sugars undergo oxidation. Vegetable acids, methylglyoxal, ethyl alcohol, and carbon dioxide, have been detected among the products of oxidation. Sugars may also be oxidized *in vitro* by hydrogen peroxide, or by permanganates. It has been found that ferrous salts can catalyze certain of these oxidations. Fructose is more readily oxidized than glucose, and the furanose form (reactive or γ-fructose) more readily than the pyranose form.

Cell sap is rarely alkaline, but it is possible that protoplasmic systems *in vivo* induce cleavages in sugars similar to those brought about by alkaline solutions *in vitro* (see pp. 187 and 302).

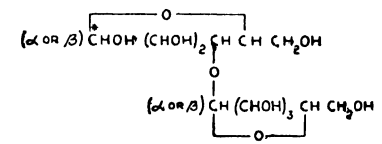
Di-, tri-, and tetra-saccharides. It is probable that di, tri,- and tetra-saccharides are produced *in vivo* by the condensation with the elimination of water of two, three, or four, monosaccharide molecules. Union takes place between hydroxyl groups in the combining molecules. In at least one of the monosaccharides the hydroxyl group attached to the aldehydic or ketonic carbon atom is, as a rule, involved. The product of the reaction is then termed a glycoside. Evidently either α-glycosides or β-glycosides may be produced by the condensation of monosaccharides.

For the production of a disaccharide from hexose sugars we may write :

$$C_6H_{12}O_6 + C_6H_{12}O_6 \underset{\text{hydrolysis}}{\overset{\text{condensation}}{\rightleftharpoons}} C_{12}H_{22}O_{11} + H_2O.$$

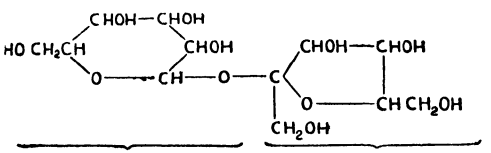
Disaccharides are easily hydrolyzed by hot dilute solutions of mineral acids (which act as catalysts) or by their specific hydrolyzing enzymes at ordinary temperatures, and the constituent monosaccharides are set free. Consequently it has been suggested that when either condensation or hydrolysis is effected *in vivo* an equilibrated state is reached (*cf.* remarks on esterification, p. 420).

The diversity which exists among disaccharides possessing the molecular formula $C_{12}H_{22}O_{11}$, may be attributed to several causes. First, the constituent hexose sugars may be different. Cane sugar or sucrose (glucose residue-O-fructose residue), is obviously different from malt sugar or maltose (glucose residue-O-glucose residue), and from melibiose (glucose residue-O-galactose residue). Cane sugar is a glucosefructoside ; maltose a glucoseglucoside ; and melibiose a glucosegalactoside.



Maltose (α-glucose-α-glucoside) or
cellobiose (β-glucose-β-glucoside).

Secondly, diversity may result from the union of different forms of the same sugar. Thus both maltose and cellobiose (glucose residue-O-glucose residue) give only *d*-glucose on hydrolysis. There are, however, good reasons for believing that maltose is an α-glucose-α-glucoside (*i.e.*, is produced by the condensation of α-glucose with itself), and that cellibiose is a β-glucoside (*i.e.*, is produced by the condensation of β-glucose with itself). It should be noted that maltose and cellobiose are reducing sugars, and will form osazones.



Glucopyranose. Fructofuranose.
Cane sugar (sucrose).

Again, hydroxyl groups differently situated in the combining monosaccharides may be involved. Since cane sugar neither reduces Fehling's solution nor forms osazones, the ketonic group of the fructose must combine with the aldehydic group of the glucose in producing the disaccharide. Turanose (glucose residue-O-fructose residue), on the other hand, although composed of the same two sugars, reduces Fehling's solution and forms osazones. Evidently free reducing groups remain uncombined in turanose.

It is an important fact to note that the fructose occurs in the reactive form in cane sugar. This disaccharide, which is widely distributed in plants, may provide a continuous supply of a reactive sugar which cannot exist for long in the free state. On hydrolysis cane sugar yields glucopyranose (normal glucose) and fructofuranose (γ-fructose). *In vitro*, the nascent γ-fructose set free is speedily converted into stable fructose, but *in vivo* it is probable that active fructose undergoes metabolic transformations.

Although the number of possible trisaccharides is even greater than that of disaccharides, very few have been found in living cells. Raffinose (galactose residue-O-glucose residue-O-fructose residue) is the best known example. In this sugar, which occurs in beet, galactose and glucose are united as in melibiose, and glucose and fructose as in sucrose. Raffinose can be hydrolyzed into its component hexose units. Under special conditions it can be split either into galactose and sucrose or into fructose and melibiose. Stachyose is a tetrasaccharide which occurs in the tubers of *Stachys tubifera*, and in various organs of other plants: it is compounded of residues of two molecules of galactose and one molecule of glucose and of fructose.

Pentosan and hexosan polysaccharides. Carbohydrates belonging to these groups may be regarded as products formed by the condensation of a very large number (n) of molecules of one or more monosaccharides. If hexose monosaccharides alone take part in the condensation, the resulting product is called a hexosan, $(C_6H_{10}O_5)n$. Pentosans, $(C_5H_8O_4)n$, are formed from pentoses, and mixed polysaccharides, *i.e.*, hexosan-pentosan complexes, are also known. On hydrolysis, polysaccharides yield the constituent monosaccharide units. Usually, however, intermediate products have at least a transient existence. It has been suggested that the interconversions of monosaccharides and polysaccharides in living cells are reversible processes:

$$(C_6H_{10}O_5)_n + (n/2)\,H_2O \underset{\text{hydrolysis}}{\overset{\text{condensation}}{\rightleftharpoons}} (n/2)\,C_{12}H_{22}O_{11}$$

$$(n/2)\,C_{12}H_{22}O_{11} + (n/2)\,H_2O \underset{\text{hydrolysis}}{\overset{\text{condensation}}{\rightleftharpoons}} n\,C_6H_{12}O_6.$$

A disaccharide containing an aldehydic or ketonic grouping can also condense with itself to give a polysaccharide. Cane sugar, however, cannot alone form the basis of a polysaccharide unit, although, of course, it might form one of the components of a polysaccharide. Actually, however, it has not yet been shown to be present in any naturally occurring polysaccharide.

Very great diversity may exist among polysaccharides possessing the general formula $(C_6H_{10}O_5)_n$, *i.e.*, the hexosans. The first point to note is that n may be very different in different substances. Evidently we cannot speak of isomerism when discussing hexosans, unless n has the same value in two different substances. It is conventional to describe as a polysaccharide any carbohydrate whose molecule is bigger than that of a tetrasaccharide. Thus there may be formed from a given monosaccharide (*a*) polysaccharides with relatively small molecules, and (*b*) polysaccharides with giant molecules, in which n may be several hundred. Because of the differences in size of their molecules, the products (*a*) and (*b*) will have very different physical properties. Again, different hexosans may be constructed from different monosaccharides. Glucosans (*e.g.*, starch, dextrin, cellulose, glycogen) give only glucose on hydrolysis; and fructosans (*e.g.*, inulin) give only fructose. The names mannan and galactan are used to describe polysaccharides which, on hydrolysis, yield mannose and galactose respectively. Mannans and galactans, or their acidic derivatives, occur with other hexosans, or with the pentosans xylan and araban, in the mixed polysaccharides found in hemicelluloses, gums, mucilages, pectic substances, etc. To such polysaccharides names such as mannogalactan, galactoaraban, glucoxylan, etc., are given, in order to indicate the nature of the constituent monosaccharides.

Even for a given value of n, it is theoretically possible for an immense number of different individuals to exist in each polysaccharide type (*e.g.*, the glucosans). For instance, a glucosan might be constructed (*a*) from α- or β-glucose, or from a mixture of the two forms, or (*b*) from either pyranose or furanose varieties of the monosaccharides. Cellulose appears to be constructed from β-glucopyranose units, since it can be converted into cellobiose by hydrolysis. Starch yields maltose on hydrolysis, and, consequently, must contain α-glucopyranose units. According to one view the amylopectin of starch grains (see below) may also contain β-glucopyranose units ; but certain authorities (see Hanes, *114*) hold that physically distinguishable starch fractions, such as amylose and amylopectin, are composed of identical molecules. These macromolecules of starch are conceived to be unbranched chain structures, each containing about thirty glucopyranose units joined together by glucosidic linkages as in maltose,[1] and each having a molecular weight of about 5,000. By association in various ways such macromolecules may produce the large sized particles which aggregate in the physically distinguishable fractions of the starch grains that are produced in the plastids of green plants.

Again, prodigious possibilities of variation result from the fact that every hydroxyl group in a monosaccharide or disaccharide is capable of participating in a condensation. It is noteworthy therefore that the naturally occurring glucosans can be classified into a small number of groups (starches, celluloses, glycogens, lichenins, etc.). This fact is evidence for the occurrence of directive synthesis in living cells (p. 412).

It is important to remember that traditional names such as starch, hemicellulose, mucilage, etc., denote well-defined plant products, which are as a rule mixtures of several chemical compounds. It has long been known that there are differences in the morphology, and in the physical and microchemical properties, of starch grains from different genera. Reichert was able to detect differences in the grains of different races belonging to the same species (see Blackman, *34*). Evidently the term starch denotes a heterogeneous group of glucosans which have certain physicochemical and physiological properties in common. Moreover, a single natural starch grain is a heterogeneous substance. Amylose, the central part, is soluble in water, and there is an insoluble husk termed amylopectin ; it has been stated that the latter component contains phosphoric acid in organic union. It has also been reported that in graminaceous starches there is another glucosan component, viz., amylohemicellulose, in which calcium, iron, and magnesium are combined with phosphoric and silicic acids. These acids are combined as esters with the carbohydrate.

The name hemicellulose denotes a polysaccharide component of cell walls, which serves as a food reserve. Hemicelluloses as a class are insoluble in water, but dissolve in dilute alkali. When hydrolyzed by weak acids they may yield pentoses in addition to hexoses. Hemicelluloses usually contain mannans and galactans, but their chemical composition is extremely variable. The products of hydrolysis of mucilages are similar to those of hemicelluloses. Mucilages are characterized, however, by their physical property of absorbing water and becoming slimy.

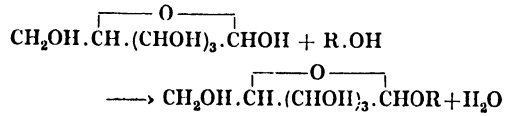
E. Glycosides

The glycosides have been divided into two sub-groups, viz., the monosides and biosides. The monosides are compounds of monosaccharides,

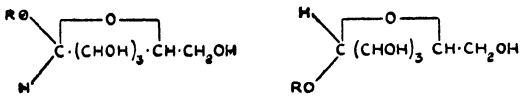
[1] The perspective drawing in fig. 2 depicting glycogen will serve also to show how it is believed glucose molecules are joined together in starch.

and include glucosides, fructosides, rhamnosides, etc. Dimonosides are compounds containing two independent monosaccharide units. The biosides are compounds of disaccharides.

The hydroxyl radical belonging to the potential aldehydic or ketonic group of a sugar is very reactive. It readily combines with other groups, particularly with a hydroxyl group in another molecule, R.OH, to give a compound called a glycoside.



α-sugars yield α-glycosides, and β-sugars yield β-glycosides.



α-Glycosides. β-Glycosides.

Many of the naturally occurring glycosides are β-glucosides.

The composition of R.OH may be very varied. Thus disaccharides arise from the glycosidal combination of two monosaccharides, and glycosides containing phenolic, alcoholic, and aldehydic, residues are widely distributed in plants. The coumarin glucosides and glycosidal anthoxanthic and anthocyan pigments, and tannins, are considered elsewhere. We only have room here to mention a few additional representatives of this varied and widely distributed class of compound.

Several of the aldehydic glucosides yield hydrogen cyanide on hydrolysis, and are, therefore, called cyanogenetic or cyanophoric glucosides. Prulaurasin, which occurs in the leaves of the cherry laurel, is racemic (*d-l-*) mandelonitrile glucoside,[1] $C_6H_5.CH(CN).O.C_6H_{11}O_5$. Prunasin, which occurs in the bark of the wild cherry, is *d*-mandelonitrile glucoside. Sambunigrin, which occurs in elder leaves, is *l*-mandelonitrile glucoside. Amygdalin, one of the best known of all glucosides, is a diglucoside of *d*-mandelonitrile, $C_6H_5.CH(CN).O.C_6H_{10}O_4.O.C_6H_{11}O_5$. It is readily extracted from bitter almonds, and is present in the kernels of most fruits belonging to the Rosaceae. On complete hydrolysis under the agency of emulsin it yields two molecules of glucose, benzaldehyde, and hydrogen cyanide (a volatile substance which turns moist sodium picrate paper brown):

$$C_{20}H_{27}NO_{11} + 2H_2O \longrightarrow 2C_6H_{12}O_6 + HCN + C_6H_5.CHO.$$

Salicin, which is widely distributed in the genus Salix, is a saligenin glucoside. When hydrolyzed under the agency of emulsin it yields saligenin, which gives a violet colour with ferric chloride:

$$C_6H_{11}O_5.O.C_6H_4.CH_2OH + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_4(OH).CH_2OH.$$

Sinigrin and sinalbin are the names given to the so-called mustard-oil glucosides that occur in the seeds of the black and white mustard respec-

[1] The formula of this nitrile of mandelic acid is $C_6H_5.\overset{*}{C}HOH.CN$. It will be noticed that one of the carbon atoms is asymmetric. Glucoside formation takes place by linkage of glucose with the hydroxyl radical in the secondary alcohol group.

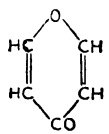
tively. On hydrolysis, *sinigrin* yields glucose, potassium hydrogen sulphate, and the pungent allyl isothiocyanate,[1] $C_3H_5.NCS$; *sinalbin* yields glucose, acid sinapin sulphate, $C_{16}H_{24}O_5N.HSO_4$, and the pungent *p*-hydroxybenzene isothiocyanate, $C_7H_7O.NCS$. It should be noted that these plants possess the power of changing the sulphate ion into the acid-sulphate ion and into the isothiocyanate ion.

F. Heterocyclic Nitrogen-Free Compounds

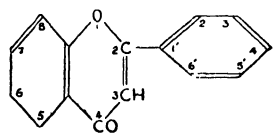
Furan and pyran rings. The furan ring, a heterocyclic ring composed of four carbon atoms and one oxygen atom, is represented in compounds which have already been discussed. It is present in furanose sugars, and occurs, fused, with a benzene nucleus, in coumarin and aesculetin.

The pyran ring, a six-membered heterocyclic ring, is present in pyranose sugars, and is represented in the molecules of the flavonic, xanthonic, and anthocyan pigments, and of certain catechin compounds.

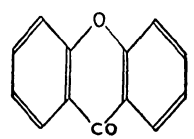
Anthoxanthin pigments. The presence of the heterocyclic nucleus (γ-pyrone) characterizes this group, which include flavonic and xanthonic pigments. It is associated with a benzene nucleus in benzo-γ-pyrone. Flavone (phenyl benzo-γ-pyrone) is a phenyl substituted derivative of this substance. Xanthone is dibenzo-γ-pyrone.



γ-Pyrone.

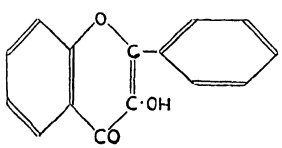


Flavone
(Phenyl-benzo-γ-pyrone).

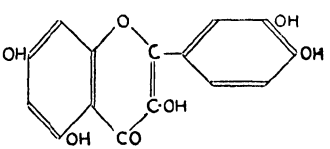


Xanthone
(Dibenzo-γ-pyrone).

In flavonol, the hydroxyl radical is substituted for the hydrogen atom attached to carbon in the pyrone ring:



Flavonol
(3-hydroxy-flavone).



Quercitin
(5 : 7 : 3′ : 4′-tetrahydroxy-flavonol).

Hydroxyl and methoxyl derivatives of flavone, flavonol, and xanthone, occur in plants either in the free state, or in glycosidal combination with the sugars, glucose and rhamnose. Chemical variation in the flavonic and xanthonic part of the molecule results from differences in the number and position of the constituent hydroxyl and methoxyl groups. By referring to the drawing of the structural formula of quercitin (5 : 7 : 3′ : 4′-tetra-hydroxy-flavonol), a flavonol found either in the free state or in a variety of glycosidal forms in oak bark,[2] the berries of Rhamnus, and in a wide range of flowers and leaves, the reader will be able to construct the formulæ

[1] *Cf.* the occurrence in other genera of sulphur with the allyl radical (p. 413).

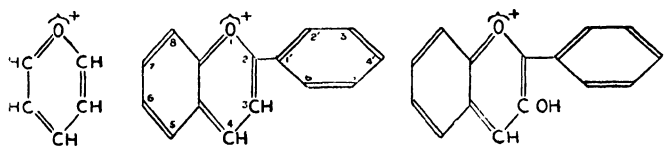
[2] The glycoside occurring in oak bark is a glucoside and is known as quercitrin. It should be noted that every glycoside is given a different name from that of its flavonic constituent.

of kaempferol (5 : 7 : 4′-trihydroxy-flavonol), fisetin (7 : 3′ : 4′-trihydroxy-flavonol), a well-known isomer of kaempferol, and myricetin (5 : 7 : 3′ : 4′ : 5′-pentahydroxy-flavonol). Flavone occurs in the free state as a mealy substance on the surfaces of several species of Primula. Chrysin, a constituent of poplar buds, is 5 : 7-dihydroxy-flavone ; apigenin, which has been found in parsley and in the flowers of the ivory-white snapdragon, is 5 : 7 : 4′-trihydroxy-flavone ; and luteolin, a pigment which has from remote antiquity been extracted from dyer's weed and dyer's broom, is 5 : 7 : 3′ : 4′-tetrahydroxy-flavone. It should be noted that two or more different flavonic substances may exist together in the same cell, and that the same flavonic substance may be produced by the metabolism of widely different genera.

All these compounds dissolve in water to give yellow solutions. The colour may be intensified by making the solution alkaline. As a rule the concentration of anthoxanthins in cell sap is too low for a yellow colour to be seen. The presence of these yellow vacuolar pigments may, however, be readily demonstrated by placing a tissue containing them in ammonia vapour. Occasionally the concentration is sufficiently high to colour the tissue yellow. For instance, luteolin and apigenin give the colour to the petals of the yellow flowered variety of *Antirrhinum majus*. It should be noted that the chemical structure of the yellow vacuolar pigments is very different from that of the carotinoids, *i.e.*, the yellow and orange plastid pigments.

The anthocyans. The soluble red and blue pigments found in the higher plants, particularly in the cell sap of flowers and fruits, were known as the anthocyans long before their chemical structure was elucidated. The term now denotes derivatives of phenyl-benzo-γ-pyrilium.[1]

Reduction of the pyrone ring gives the heterocyclic ion, γ-pyrilium which is present in the anthocyan pigments. Phenyl-benzo-γ-pyrilium is evidently a reduction product of flavone. Reduced flavonol is 3-hydroxy-phenyl-benzo-γ-pyrilium. These and other reduced flavones and flavonols are known as anthocyanidins.



γ-Pyrilium. Reduced flavone (Phenyl-benzo-γ-pyrilium). Reduced flavonol.

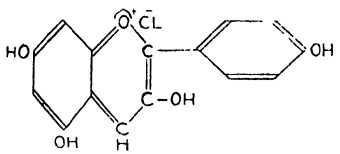
It should be noticed that reduction leads to a shifting of valency bonds. The residual oxygen atom acquires a positive charge, which enables it to hold a negatively charged ion by electrostatic attraction. Consequently these pyrilium compounds readily form oxonium salts (*e.g.*, oxonium chlorides) with acids.

Nearly all the natural anthocyan pigments are reduced flavonol glycosides. Recently, however, an anthocyan referable to the flavone, apigenin, has been found in flowers of *Gesnera fulgens*. These red and blue vacuolar pigments, when in the glycosidal state, are called anthocyanins. The

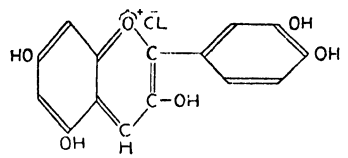
[1] It has been reported that the *yellow* pigment which is present in the flowers of certain varieties of *Papaver alpinum* is a phenyl-benzo-γ-pyrilium derivative. Accordingly, in spite of its colour, it must on chemical grounds be included in the class, anthocyan.

pigmented component, anthocyanidin, and the combined sugar are set free by acid hydrolysis. Anthocyanins are insoluble in amyl alcohol, and, in consequence, are readily distinguished from the anthocyanidins, which are soluble in this solvent.

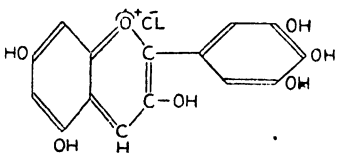
Variation in the chemical structure of anthocyanins is similar to that of the flavonic pigments, and results from differences in the number and position of the hydroxyl or methoxyl groups [1] that are substituted for hydrogen in the anthocyanidin component of the molecule. This similarity is clearly seen when the chemical structure of the anthocyanidins, pelargonidin, cyanidin, and delphinidin is compared with that of the corresponding flavonols. Pelargonidin is a reduction product of kaempferol; cyanidin is a reduction-product of quercitin; and delphinidin is a reduction product of myricetin.



Pelargonidin chloride.

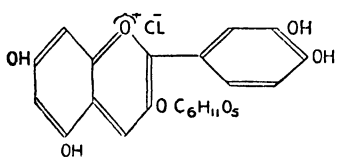


Cyanidin chloride.

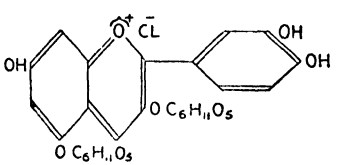


Delphinidin chloride.

The anthocyanins may be classified in terms of their constituent anthocyanidins. For instance, the pelargonidin anthocyanins, the cyanidin anthocyanins, and the delphinidin anthocyanins, constitute distinct groups. In each group variation results from differences in the nature of the sugar represented in the molecule. Thus glucose, galactose, rhamnose, and disaccharides, are represented in anthocyanins. Moreover, anthocyanins may be monoglycosides or diglycosides. It appears from the achievements of Robinson and his co-workers on the synthesis of anthocyanins that, in monoglycosides, the sugar preferentially enters into glycosidal combination with the hydroxyl group in position 3 in the anthocyanidin molecule. In diglycosides the second sugar residue is found combined in position 5 of the anthocyanidin molecule.



Chrysanthemin chloride.



Cyanin chloride.

[1] As an example of an anthocyanidin containing methoxyl groups, we cite œnidin, which is represented in œnin, the pigment in the vacuoles of the epidermal cells of the blue grape.

The cyanidin group of anthocyanins may conveniently be selected for further discussion. There are many distinct anthocyanins known which give on hydrolysis the anthocyanidin, cyanidin, and one or more sugars. We may cite cyanin [1] and chrysanthemin as examples. The structural formulæ of the chlorides are figured above. We note that chrysanthemin is a 3-monoglucoside of cyanidin, and cyanin a 3-5-diglucoside of cyanidin. Chrysanthemin occurs in the genus Chrysanthemum. Cyanin is found in the blue cornflower, in the magenta *Rosa gallica*, and in certain varieties of Dahlia. It is noteworthy that in widely different genera the protoplasmic system which determines anthocyanin formation, *i.e.*, anthocyanidin and glycoside formation, may be identical. It should also be noted that two distinct anthocyanins may be produced in the same flower; for example, asterin (a cyanidin anthocyanin) and callistephin (a pelargonidin anthocyanin) have been found in the petals of the aster.

Attempts have been made (see Onslow, *215*, and Scott-Moncrieff, *263*) to describe in chemical terms the genetical concepts that have proved useful in explaining the production of different colour varieties of flowering plants. Clearly, the first problem is to elucidate the sequence of biochemical changes that give rise to an anthocyanidin. Evidence exists that anthocyanin formation is promoted in certain leaves either as a result of feeding them with a sugar or under conditions that may lead to the accumulation of sugars in the leaves. It should be noted that flavones, flavonols, and anthocyanidins contain fifteen carbon atoms. Robinson has suggested that two molecules of hexose with a cleavage product containing three carbon atoms are concerned in the production of a phenolic common precursor of these compounds. In support of this suggestion he states that true anthocyanidins can be produced *in vitro* by treating with strong boiling hydrochloric acid colourless extracts of flowers, leaves, seeds, etc., that contain certain phenolic substances. To one of these substances he gave the name, peltogynol. At one time it was thought that flavonic substances might be the precursors of anthocyanidins. Scott-Moncrieff does not favour this hypothesis of sequential synthesis, because she has obtained evidence that the production of flavonic compounds may lead to a partial suppression of anthocyanidin formation. For this and for other reasons she suggests that flavonic and anthocyan pigments "are formed at least in part from a common but limited source, but by parallel rather than by sequential synthesis."

Among the other matters discussed by Scott-Moncrieff are the genetical relationships of such anthocyanins as may differ from one another in molecular structure because they contain different anthocyanidins, or because there is variation in the nature of or in the position occupied by the sugar residues in the molecules of the pigments. She also considers at length the important question of the colour shown by a given anthocyanin when dissolved in cell sap.

Robert Boyle recognized that anthocyanins are natural indicators. They are red in acid solutions and many of them change colour through violet to blue as the acidity decreases. The change from red to blue may be shown by anthocyanins at *pH* values that are less than 7 (Buxton and Darbishire, *57*, and Smith, *273*). For example the extracted sap of *Salvia patens* is pink at *pH* 3 and blue at *pH* 4; whereas that of Aconitum does not turn blue until the *pH* is greater than 6. The anthocyanins belonging to the

[1] It should be noted that the names given to anthocyanidins end with the suffix -idin, and those of the anthocyanins with the suffix -in (compare, for example, cyanidin and cyanin).

so-called clear-red types of flower (*e.g.*, *Salvia splendens*) change from vermilion to purple or brown, without giving any blue colour. Willstätter suggested that the red colour represents an anthocyanin existing as an acid salt, and the blue colour a potassium or some other metallic phenolate, and that the violet anthocyanin is an anhydride.

Until recently variation in the colour of the flowers of different varieties, species, or genera, containing the same anthocyanin, was entirely attributed to differences in the acidity of the vacuolar sap in cells of the petals. Robinson and Robinson (*251*) have, however, shown that co-pigments, which can modify the colour of a given solution of anthocyanin, are frequently present in cell sap. It has long been known that traces of salts of iron, aluminium, and other metals, may, at a constant *pH*, bring about a change from red to blue in the colour of solutions of certain anthocyanins. Robinson and Robinson discovered that organic substances also can act as co-pigments. They observed the colour change from red to blue brought about when various substances were added to a solution of œnin chloride in dilute hydrochloric acid. They state that tannins, and flavonic substances are the chief natural co-pigments. In general they conclude that " great changes in the colour of varieties in a species are not brought about by changes in the *pH* of the cell sap, but rather by changes in the nature of the anthocyanin, including in their train the formation of complexes with organic substances and possibly with metals such as iron." They also point out that blue colours may sometimes result because anthocyanins are colloidally dispersed instead of being in true solution. To such colloidal effects they attribute the fact that the sap of the blue cornflower is more acid than that of the red rose, which contains in true solution the same anthocyanin, viz., cyanin. It should be noted, however, that Scott-Moncrieff mentions that in a few instances higher *pH* values may result in bluer toned varieties.

PART II. ORGANIC COMPOUNDS CONTAINING NITROGEN

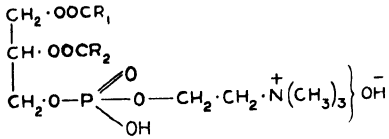
The majority of the nitrogenous metabolic products may be regarded as substitution derivatives of one or more molecules of ammonia,[1] *i.e.*, as mono-, di-, tri-, or polyamino compounds. These may be primary amines, RNH_2, secondary amines $(R)_2NH$, tertiary amines $(R)_3N$, or quarternary ammonium bases $(R)_4NOH$. We shall consider these products under the headings (*a*) open-chain compounds (other than amino acids and proteins), (*b*) heterocyclic compounds, (*c*) proteins, amino acids, and amides.

A. Naturally occurring Open-chain Amines

It is known that the simple aliphatic amines occur in the free state in plants, but they have not been much investigated. Methylamine has been found in dog's mercury, and trimethylamine in *Chenopodium vulvaria* and in the flowers of the common hawthorn and the pear.

[1] We have already noted that substances containing the cyanogen radical are also found in plants (p. 434). There is no evidence of the occurrence of nitro- or nitroso-organic compounds in living organisms. Moreover, amino-groups are not found substituted for hydrogen in the benzene ring. It should be noted that certain classes of compounds well known to the organic chemist are not produced by plant metabolism.

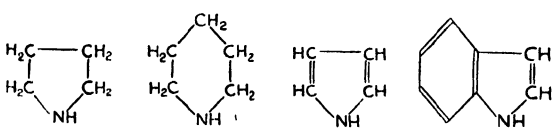
The lipoids or lecithins, which are important constituents of protoplasm, belong to this group of compounds. They may occur in the free state or as lipoproteins. The lecithins, like fats, are soluble in ether, chloroform, etc. On hydrolysis they yield fatty acids, glycerol, phosphoric acid, and choline (*i.e.*, ethanol trimethyl-ammonium hydroxide, $HO.CH_2.CH_2.N(CH_3)_3OH$). Accordingly the following general graphic formula has been suggested for a lecithin substance :—



A lipoid or lecithin substance.

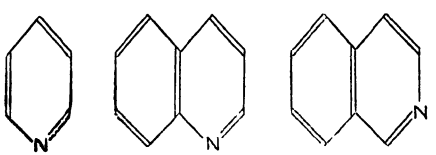
B. Naturally occurring Compounds containing Nitrogenous Heterocyclic Rings

Introductory notes. Pyrrolidine, piperidine, and pyrrole, are heterocyclic secondary amines in which the nitrogen atom is united to carbon atoms within the ring. Indole may be regarded as a derivative either of benzene or of pyrrole.



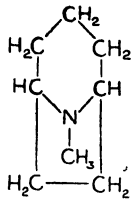
Pyrrolidine. Piperidine. Pyrrole. Indole.

Pyridine is a heterocyclic tertiary amine in which the nitrogen atom is united to carbon atoms within the six-membered ring. A monocarboxylic acid of pyridine, which can be obtained by the oxidation of nicotine, is known as nicotinic acid. Its amide is of great physiological interest (p. 444). Pyridoxin (vitamin B_6) has been obtained from cereal grains. Its chemical name is 2-methyl, 3-hydroxy, 4-5 dihydroxymethyl pyridine. Evidently piperidine is a reduction product of pyridine. Quinoline and isoquinoline may be regarded as derivatives either of benzene or of pyridine.



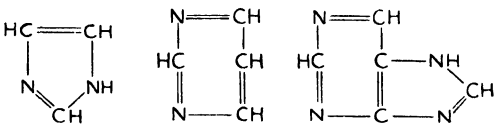
Pyridine, Quinoline. Isoquinoline.

Tropane is a tertiary amine which may be regarded as a derivative either of piperidine or of pyrrolidine.



Tropane,

Di-, tri-, and polyamines, occur among plant products. Pyrimidine and iminazole are heterocyclic diamines, and derivatives of these compounds are present in all living cells.



Iminazole. Pyrimidine. Purine.

Substituted purines are also essential components of protoplasm. Purine itself may be regarded as a derivative either of pyrimidine or of iminazole.

Pyrrole derivatives. (i) *Porphyrin compounds.* Porphyrins are built up from four substituted pyrrole nuclei, which are united through carbon atoms. Porphyrin types vary according to the nature of the groups which substitute hydrogen atoms in the pyrrole nuclei. Metals can combine with the nitrogen atoms in the pyrrole nuclei giving metallo-porphyrins. Thus hæmatin, which can be derived from the hæmoglobin of red blood corpuscles, is an iron porphyrin compound, whereas chlorophylls *a* and *b* are magnesium porphyrin compounds. Bacteriochlorophyll, the green pigment of the Thio- and Athiorhodaceae (p. 249), has a very similar structure to that of chlorophyll *a*, but contains two more hydrogen atoms per molecule; consequently one of the double bonds in one of the pyrrole rings becomes a single bond; also it contains an acetyl group ($CH_3.CO-$) in place of the vinyl group ($CH_2=CH-$).

It is now established that hæmatin derivatives are present in the living cells of all aerobic organisms (see Keilin, *164*). These iron-containing porphyrin derivatives exist in combination with nitrogenous organic substances, and the conjugated compounds are called the hæmochromogens. They play an important part in cellular oxidations (see chap. III, section D). Here we recognize the probability that the cleavages, oxidations, reductions, condensations, and other changes which lead to the production of the porphyrin component of the cytochromes, peroxidase, catalase, etc., do not greatly differ in type from those which bring about the formation of the chlorophylls.

In the solid state, chlorophyll *a* is a bluish-black powder which dissolves easily in most organic solvents, but is only sparingly soluble in petroleum ether. In the solid state chlorophyll *b* is a dark green or greenish-black glistening powder, which is quite insoluble in petroleum ether, and, in general, dissolves less readily than chlorophyll *a* in organic solvents. When dissolved in these solvents both varieties show a red fluorescence. Colloid solutions in water show no fluorescence (see p. 227).

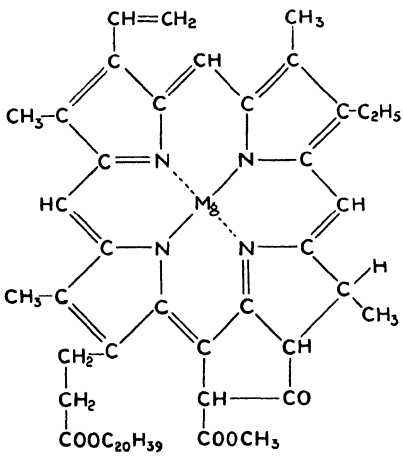
The molecular formulæ of chlorophyll *a*, $C_{55}H_{72}O_5N_4Mg$, and chlorophyll *b*, $C_{55}H_{70}O_6N_4Mg$, indicate that they are closely related compounds.

Evidently chlorophyll *b* may be regarded as an oxidation product of chlorophyll *a*. Analysis has shown that chlorophyll *a*,

$$(MgN_4C_{32}H_{30}O)\,(COOCH_3)\,(COOC_{20}H_{39}),$$

and chlorophyll *b*, $(MgN_4C_{32}H_{28}O_2)\,(COOCH_3)\,(COOC_{20}H_{39})$, are methyl phytyl esters of two acids respectively called chlorophyllin *a* and chlorophyllin *b*. The term phytyl refers to the hydrocarbon residue of phytyl alcohol or phytol, $C_{20}H_{39}OH$ (see p. 417).

By hydrolysis with cold alkali, the alkaline salts of chlorophyllin *a* and chlorophyllin *b* are obtained.[1] When these salts are heated with alkali, carbon dioxide is eliminated, and the product can be converted into the metallo-porphyrin derivative called ætiophyllin, $C_{32}H_{34}N_4Mg$. This oxygen-free product may be regarded as the central structure of the chlorophyll molecule. By the action of mineral acids on ætiophyllin, the atom of magnesium is eliminated, and ætioporphyrin is produced. On the basis of these and other experimental results, the following formula has been suggested for chlorophyll *a* :—



Chlorophyll *a*.

(ii) *Indole compounds.* Indole, C_8H_7N, which, as we have already pointed out, contains a pyrrole ring, is found combined in tryptophane (see

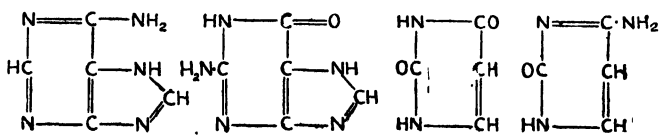
[1] This fact may be exploited to show the presence of green and yellow pigments in chloroplasts. All the pigments are first extracted from dried leaves (Willstätter used dried nettle leaves) with 80 per cent. acetone. By gently shaking the resulting green acetone-solution with ether, an ethereal solution of all the pigments is obtained. Hydrolysis is effected by using a strong solution of caustic potash in methyl alcohol. Phytol and methyl alcohol are set free, and the potassium salts of the chlorophyllin acids are produced. These green potassium salts may now be extracted with water, in which they are soluble. The yellow pigments (carotin and xanthophyll), being insoluble in water, remain in the ether layer. The methods used for separating carotin from xanthophyll, and chlorophyll *a* from chlorophyll *b*, depend upon the fact that each pair of substances shows differential solubility in mixed solvents (for details see Onslow, *216*). It should be noted that Tswett, by his chromatographic method, obtained from a solution of plastid pigments a chromatogram consisting of two green as well as yellow pigments.

p. 446). As indoxyl or hydroxy-indole, $C_8H_6N(OH)$, this heterocyclic ring occurs in the glucoside indican, $C_8H_6N\text{-}O\text{-}C_6H_{11}O_5$. Since prehistoric times indigo has been prepared from extracts of *Isatis tinctoria* (woad), species of Indigofera, and other plants containing indican. This dye is formed by the oxidation and condensation of indoxyl molecules which are liberated by the hydrolysis of indican.

The alkaloids. The alkaloids are vegetable bases, which possess heterocyclic rings, and exert physiological effects on animals. They may occur in combination with vegetable acids. In recent years the structure of many of these complex compounds has been established by synthesis. The alkaloids may be regarded as derivatives of pyridine, pyrrolidine, tropane, quinoline, and isoquinoline. There are only a few records of the occurrence of alkaloids outside the dicotyledons. In this group of plants they have, for example, been found in the Solanaceae, Papaveraceae, Leguminosae, and Umbelliferae, but they are not widely distributed among the various families. The structural formulæ of four important alkaloids are given below.

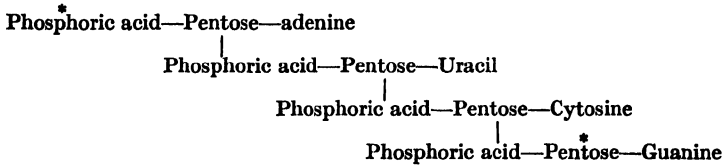
Nicotine, which occurs in the leaves of *Nicotiana tabacum*, is both a pyridine and a pyrrolidine alkaloid ; cocaine, which is obtained from the leaves of *Erythroxylum coca*, is a tropane alkaloid ; quinine, which is found in the bark of certain species of Cinchona, is a quinoline alkaloid ; and narcotine is an isoquinoline alkaloid.

Iminazole-, pyrimidine-, and purine-derivatives. Iminazole is represented in the amino acid histidine (see p 446). Derivatives of pyrimidine and purine are present in yeast or plant nucleic acid (ribonucleic acid), and thymus or animal nucleic acid (desoxyribonucleic acid). It is now known that these two nucleic acids occur in both plant and animal cells and there is evidence, summarized by Davidson and Waymouth (*72*), that it is the desoxyacid which links with proteins in the chromosomes of the nucleus. On complete hydrolysis ribonucleic acid yields phosphoric acid, *d*-ribose, purine bases (guanine and adenine), and pyrimidine bases (cytosine and uracil). The desoxyacid yields desoxyribose instead of ribose (p. 427), and thymine (methyluracil) instead of uracil.



Adenine. Guanine. Uracil. Cytosine.

Levene has suggested that the component molecules of yeast nucleic acid are united thus :—



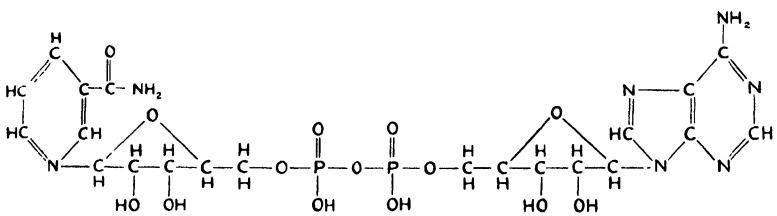
Alternatively union of the asterisked molecules of phosphoric acid and pentose may result in a ring structure. It is probable that the pentose and phosphoric acid components are united as in the organic phosphates. The pentose and either the purine or the pyrimidine are combined as glycosides,

called nucleosides. By the hydrolysis of nucleic acid nucleotides are, however, first formed. A nucleotide is a compound derived from phosphoric acid, a sugar, and a pyrimidine or a purine. Hydrolysis of a nucleotide yields phosphoric acid and a nucleoside. Then finally the nucleoside is cleaved. All these cleavages have been effected *in vitro* by enzymes.

Purines have also been found in the free state in plants. For example, adenine, theobromine, and caffeine, occur together in the leaves of the tea plant. Theobromine and caffeine are oxidation products of methyl derivatives of purine.

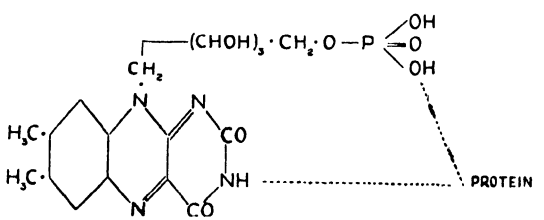
Nucleotides and allied substances. The four nucleotides occurring combined in nucleic acid are known as adenylic acid, uridylic acid, cytidylic acid, and guanylic acid. In the free state adenylic acid possesses the properties of a cophosphorylase (p. 50). No functions have as yet been assigned to the other nucleotides.

In recent years the chemical structure has been established of certain other coenzymes, which show structural affinities to nucleotides. Thus codehydrase I (cozymase) is a double nucleotide ; one part of the molecule is a pyridine nucleotide, and the other an adenine nucleotide. The whole molecule was described as diphosphopyridine nucleotide (p. 38) before it was known that it contained a second ribose residue and an adenine residue. It should be noted that codehydrase II has a similar structure, but contains three molecules of phosphoric acid (p. 38). The pyridine residue in these two dehydrases is nicotinic acid amide (p. 440). In lists of vitamins this amide is described as the Pellagra Preventive (PP) factor.



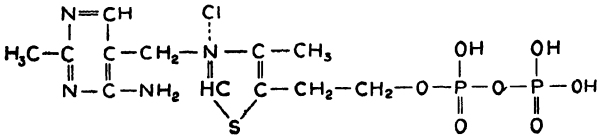
Codehydrase I (cozymase).

Codehydrases are the prosthetic groups (p. 450) of certain holodehydrases (p. 37). The prosthetic group of the yellow enzyme (p. 39) is referred to as riboflavin phosphoric acid and as alloxazine nucleotide. It is composed of the complex alloxazine ring, ribose, and phosphoric acid. In animal nutrition this prosthetic substance shows properties belonging to one of the components of the vitamin B_2 complex. It is a growth factor for certain microorganisms. A suggested structural formula for the whole enzyme is figured below.



Yellow enzyme.

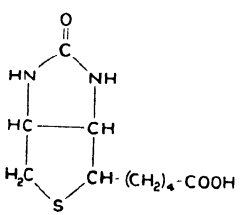
The molecule of cocarboxylase (pp. 50 and 60), the structure of which is figured below, is evidently not that of a nucleotide, since it does not contain a sugar residue. It resembles a nucleotide, however, in that it is a phosphoric ester, and contains a pyrimidine ring. The presence of the thioazole ring in the molecule is noteworthy. This ring contains the essential element sulphur as well as carbon and nitrogen. The suggestion that the nitrogen in this ring is able to combine with chlorine is interesting; but it is not yet established that chlorine is necessarily present in the cocarboxylase molecule.



Cocarboxylase.

Cocarboxylase may be described as pyrimidine-thioazole-diphosphoric ester. Alternatively it may be called aneurin diphosphate. The name aneurin refers to that part of the molecule which is composed of the pyrimidine and thioazole rings. Great interest attaches to aneurin, because it has the properties of vitamin B_1, which corrects certain nervous disorders in animals, and because there exists some evidence that it may act in plants as a hormone in promoting the growth of roots (p. 383). It is a growth factor for certain fungi (e.g. Phycomyces) and bacteria. It should be noted that the word thiamin, which is used by many authors, is a synonym of aneurin.

Biotin, which is called vitamin H by students of animal nutrition, and bios II when it is considered as a growth factor in yeast (p. 380), is, like



Biotin.

thiamin, a heterocyclic compound containing carbon, nitrogen and sulphur atoms in a ring. Its chemical name is 2-keto-3, 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid. It has been synthesized.

C. Proteins and their Derivatives

Aminoacids. The aminoacids, $R.CH(NH_2).COOH$, produced by the hydrolysis of proteins, are derivatives of aliphatic acids in which one of the hydrogen atoms attached to the α-carbon atom is replaced by an amino group. Except in glycine, this carbon atom is asymmetric. The amino acids that occur in nature are lævorotatory. A list of the principal amino acids is given below :—

Glycine (α-amino-acetic acid) $CH_2NH_2.COOH$.
Alanine (α-amino-propionic acid) $CH_3.CH(NH_2).COOH$.
Valine (α-amino-isovaleric acid) $CH(CH_3)_2.CH(NH_2).COOH$.
Leucine (α-amino-isocaproic acid) $CH(CH_3)_2.CH_2.CH(NH_2).COOH$.
Isoleucine (α-amino-β-methyl-β-ethyl-propionic acid)

$$CH(C_2H_5)(CH_3).CH(NH_2).COOH.$$

Serine (α-amino-β-hydroxy-propionic acid) $CH_2OH.CH(NH_2).COOH$.
Aspartic acid (α-amino-succinic acid) $COOH.CH_2.CH(NH_2).COOH$.
Glutamic acid (α-amino-glutaric acid) $COOH.CH_2.CH_2.CH(NH_2).COOH$.
Ornithine (α-δ-diamino-valeric acid) $CH_2(NH_2).(CH_2)_2.CH(NH_2).COOH$.
Arginine (δ-guanidine-α-amino-valeric acid)

$$HN = C(NH_2) - NH.CH_2.(CH_2)_2.CH(NH_2).COOH$$

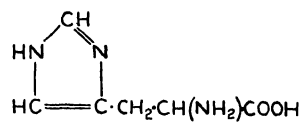
Lysine (α-ε-diamino-caproic acid) $CH_2(NH_2).(CH_2)_3.CH(NH_2).COOH$.
Cystine (di-β-thio-α-amino-propionic acid) $S.CH_2.CH(NH_2).COOH$
$S.CH_2.CH(NH)_2.COOH$.
Phenyl alanine (β-phenyl-α-amino-propionic acid)

$$C_6H_5.CH_2.CH(NH_2).COOH.$$

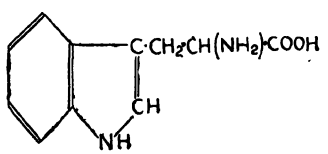
Tyrosine (β-*p*-hydroxy-phenyl-α-amino-propionic acid)

$$(HO).C_6H_4.CH_2.CH(NH_2).COOH.$$

Histidine (β-iminazol-α-amino-propionic acid)



Tryptophane, β-indole-α-amino-propionic acid



It will be observed that considerable diversity of molecular structure exists amongst these acids. Amino acids may be substitution products of aliphatic acids having either an odd or an even number of carbon atoms, and having branched chains (*e.g.*, valine, leucine, iso-leucine), or straight chains[1] (*e.g.*, alanine, lysine). Ornithine, arginine, and lysine, contain more than one amino group and are consequently more basic than the other amino acids, while aspartic and glutamic acids each contain two carboxylic groups and one amino group. Serine, cystine, phenylalanine, tyrosine, histidine, and tryptophane, are substitution derivatives of alanine. Cystine may exist in the reduced form, cystëine,

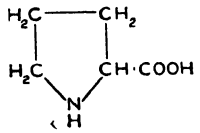
$$CH_2(SH).CH(NH_2).COOH.$$

Phenylalanine and tyrosine are aromatic compounds, and histidine and tryptophane are heterocyclic compounds.

Proline, or α-pyrrolidine-carboxylic acid, which is not an amino acid, is

[1] *Cf.* the aliphatic acids in fats. These acids have straight chains only, and contain an even number of carbon atoms.

usually present among the products of the hydrolysis of proteins (table XVII).



Proline.

Simple proteins, proteoses, peptones, and peptides. Proteins, like polysaccharides and certain other anabolic products, possess giant molecules. For instance, it has been estimated that the molecular weight of edestin, the reserve protein of hemp seed, is 208,000, and that the radius of the molecule is 3·9μμ. When hydrolyzed in the presence of acids or of enzymes, proteins yield derivatives possessing smaller molecules, viz., proteoses, peptones, polypeptides, dipeptides, and amino acids. The percentages of various amino acids, proline, and ammonia, which have been obtained by the hydrolysis of certain seed proteins are given in table XVII.

TABLE XVII. *The percentage of various amino acids, proline, and ammonia, found among the products of hydrolysis of certain vegetable proteins.*

	Edestin from hemp seed	Gliadin from wheat grains	Legumin from the pea.	Legumelin from the pea
Glycine . .	3·8	—	0·4	0·5
Alanine . .	3·6	2·0	2·1	0·9
Valine . .	—	3·3	—	0·7
Leucine . .	20·9	6·6	8·0	9·6
Proline . .	1·7	13·2	3·2	4·0
Phenyl alanine.	2·4	2·3	3·8	4·8
Aspartic acid .	10·2	0·8	5·3	4·1
Glutaminic acid	19·2	43·0	17·0	13·0
Serine . .	0·3	0·1	0·5	—
Tyrosine .	2·1	3·3	1·6	1·6
Cystine . .	1·0	1·6	0·8	—
Lysine . .	2·2	1·2	5·0	3·0
Histidine. .	2·1	2·2	1·7	2·3
Arginine . .	15·8	3·0	11·7	5·5
Tryptophane .	2·5	1·4	1·8	—
Ammonia . .	1·9	5·2	2·0	1·3

It is generally held that proteins and their derivatives result exclusively from the union of amino acids by means of peptide linkages (-CO.NH-), thus :—

$$H_2N.CH(R_1).COOH + H_2N.CH(R_2).COOH \longrightarrow H_2N.CH(R_1).CO.NH.CH(R_2).COOH + H_2O.$$

Bergmann (see *28*) has obtained evidence of the enzymic synthesis of peptides from amino acids and has maintained that in living cells the formation of proteins by the condensation of amino acids, and the hydrolysis of proteins are reversible reactions.

Proteins $\rightleftharpoons$ proteoses $\rightleftharpoons$ peptones $\rightleftharpoons$ polypeptides $\rightleftharpoons$ dipeptides $\rightleftharpoons$ amino-acids.

$\longrightarrow$ hydrolysis.

$\longleftarrow$ condensation with the elimination of water.

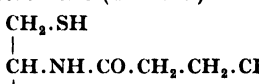
Since the properties of polypeptides, peptones, proteoses, and proteins, are determined by the nature, number and arrangement of the constituent amino acids, immense possibilities of variation exist. Theoretically an unrestricted number of proteins, considered as chemical rather than biochemical products, might be constructed from the amino acids listed above. It was at one time held that the composition of naturally occurring proteins varied indefinitely. Bergmann (see *e.g.*, *28*) has, however, obtained evidence that living organisms synthesize only such proteins as obey certain quantitative rules. One of these rules is that the total number of amino acids in the molecule must be $2^n \times 3^m$, where n and m are whole numbers. For example, cattle fibrin is composed of 576 (*i.e.*, $2^6 \times 3^2$) amino acid residues, and silk fibroin of 2,592 (*i.e.*, $2^5 \times 3^4$) amino acid residues. This idea of the limitation to definite size groups of single giant molecules of native proteins accords well with Svedberg's finding that the particle size of such protein units is a whole number multiple of 17,500. Within the possible size groups, however, the number of possible protein individuals by far exceeds the number of living organisms.

The results recorded in table XVII show that considerable differences in composition are found among the reserve proteins of seeds. Furthermore, there is definite evidence of differences in the properties of the physiologically active proteins of the cytoplasm and nuclei of different species, and indeed of different races of the same species.

The occurrence and physical properties of the simple proteins, and their derivatives. The classification of the *simple proteins* depends upon their solubilities. *Albumins* are soluble in water. *Globulins* are insoluble in water, but dissolve in dilute solutions of certain salts. *Prolamins* are insoluble in water and saline solutions, but dissolve in seventy per cent. ethyl alcohol. *Glutelins* are insoluble in water, saline solutions, seventy per cent. alcohol, but dissolve in dilute alkalies.

Albumins and globulins occur in protoplasm and as reserve food in storage tissue. Prolamins are only found in cereal grains, where they are associated with glutelins, albumins, and globulins. The prolamin of wheat is called gliadin. The gluten produced when wheat flour is mixed with water owes its tenacity to the physical properties of gliadin.

Colloid solutions termed emulsoid sols (p. 454) are obtained by dissolving proteins in water, saline solution, or some other solvent. It is probable that proteins are present in colloidal solution in cell sap. Amino acids yield crystalloid solutions. Many of these acids have been detected in the free state in plant cells. The physical properties of solutions in which proteins are undergoing hydrolysis gradually change as the dimensions of the solute molecules diminish. Thus the solution containing proteoses has been described as semicolloid, because proteoses can diffuse across a parchment membrane (p. 452) but are precipitated by saturating the solution with ammonium sulphate. Proteoses, peptones, and peptides have been detected in plant cells. The tripeptide glutathione, or γ-glutamyl-cysteinylglycine, which is formed by the union of glutaminic acid, cysteine, and glycine, plays a part in cellular oxidations (p. 40). Two molecules of the tripeptide (G-SH) may undergo dehydrogenation, and give oxidized glutathione (G-S-S-G).



Glutathione.

It is convenient to mention at this stage that pantothenic acid, which belongs to the vitamin B complex, and is a growth factor for some microorganisms, is probably present in all living plant cells, and contains a peptide linkage. It is a derivative of a substituted butyric acid and β-alanine, which is not listed among the amino acids occurring in proteins. Its full name is α-γ-dihydroxy-β,β-dimethylbutyryl-β-alanide, and its formula

$$CH_2(OH).C(CH_3)_2.CHOH.CO.NH.CH_2.CH_2COOH.$$

Since they contain amino groups as well as carboxyl groups, proteins and their derivatives may act either as bases or as acids.

Upon adding an acid, HX, to an amino acid, a salt, which in solution gives amino acid cations, is produced :

$$R.CH(NH_3X).COOH \rightarrow R.\overset{+}{C}H(\overbrace{NH_3}).COOH + X^-$$

while the metallic salt resulting from the combination of an amino acid with a base, MOH, gives amino acid anions :

$$R.CH(NH_2).COOM \rightarrow M^+ + R.CH(NH_2).COO^-.$$

Proteins and their derivatives are described as amphoteric electrolytes or ampholytes, because they can exist either as positively charged ions or as negatively charged ions. The *pH* of the solution determines whether the ions will, under the influence of an electric current, migrate to the anode or the cathode. For every ampholyte there exists a *pH* at which the number of cations of the substance is equal to the number of anions, and consequently migration in an electric field is not apparent. This important *pH* value is termed the isoelectric point of the ampholyte.

At their isoelectric points proteins show their minimum solubility and are frequently precipitated. This precipitation is reversible. On making the isoelectrical protein solution either more alkaline or more acid, the protein again dissolves. Solutions of albumins and of globulins are rapidly coagulated at 100° Centigrade. The coagulation of plant globulins is as a rule incomplete. The maximum coagulation is shown at the isoelectric point of the dissolved protein. This form of precipitation is irreversible.

Dissolved proteins are precipitated by strong solutions of certain salts (*cf.* the salting-out of the dispersed solutes of other emulsoid sols, see p. 454). Ammonium sulphate is a convenient salt to use for this purpose. Albumins are often precipitated in half-saturated solutions of ammonium sulphate. Solutions of globulins must usually be saturated with ammonium sulphate before the protein is precipitated. Such precipitation is reversible. Upon diluting the solution the protein again dissolves. Proteins are also reversibly precipitated by alcohol. If, however, the precipitate is left in the alcoholic solution, the protein gradually undergoes a change called denaturation, and becomes insoluble in water or dilute saline. The proteins are denatured in protoplasm that has been fixed by absolute alcohol. Dissolved proteins may slowly undergo spontaneous denaturation at ordinary temperatures. It is possible that certain changes in the protoplasm of old cells may be the result of the slow denaturation of the proteins.

Proteins may be irreversibly precipitated by certain chemical reagents. Acids with heavy anions (*e.g.*, tannic, osmic, picric, phosphotungstic acids), and the salts of heavy metals (*e.g.*, copper, lead, barium, mercury) react chemically with proteins and form insoluble products. The reasons why

most of these substances are poisons is therefore not far to seek. It should be noted that some of these precipitating agents enter into the composition of well-known fixatives for protoplasm.

Conjugate proteins. All living cells contain proteins which exist either in chemical combination or in physical association with other compounds, which are described as the prosthetic groups of the protein. Such complex units are termed conjugate proteins. Some authors describe them as proteids, *i.e.*, a proteid consists of a protein and a prosthetic group. The chromatin material in the nuclei of living cells is largely composed of nucleoprotein. It has been suggested that a molecule of this nucleoprotein contains two distinct proteins, A and B, in association with desoxynucleic acid. In the nucleoprotein occurring in cytoplasm, ribonucleic acid is combined with protein. In the presence of the gastric juice of animals (*i.e.*, pepsin in decinormal hydrochloric acid) protein A is split off from nucleoprotein, leaving [protein B—nucleic acid] as the residual conjugate protein. This residue is called nuclein. The insolubility of nuclein in the gastric juice should be noted. As a result of the hydrolysis of nuclein in the presence of the pancreatic juice of animals (*i.e.*, trypsin in alkaline solution), protein B and nucleic acid are set free. Since it appears that the composition of desoxynucleic acid does not vary from plant to plant, the variation in the composition of chromatin, the so-called material basis of inheritance has been attributed to variation in the composition of the protein constituents of nucleoproteins (*cf.* the remarks made on p. 448).

It is possible that protein and lipoid become associated in all living cells as lipoprotein (or lecithoprotein). According to Lubimenko (see Priestley's review, *236*) natural chlorophyll is a pigmented conjugate protein, and chlorophylls *a* and *b*, carotin, and xanthophyll, are decomposition products of this chromoprotein. Evidence supporting this view has been obtained (p. 227). It seems to be well established that phycoerythrin, the red colouring matter that occurs in the chloroplasts of plants belonging to the red algæ, is a chromoprotein.

Certain oxidation enzymes (*e.g.*, catalase, alcohol dehydrase, yellow enzyme) are conjugate proteins. Crystalline proteins have been prepared from some of these enzymes, and the structure of some of the prosthetic groups has been established by synthesis (chap. III, section D). The structural formula figured on p. 444, suggests a possible method of attachment of alloxazine nucleotide (the prosthetic group) to protein in yellow enzyme.

Amides. The chemical reaction by which amides, $R.CONH_2$, are produced from organic acids by the substitution of an amino group for a hydroxyl group is called amidation. It is probable that some of the constituent amino acids in proteins are amidated. These acid amides, $R.CH(NH_2).CONH_2$, on hydrolysis are deamidated, and yield ammonia and amino acids. Asparagine, $COOH.CH_2.CH(NH_2).CONH_2$, the amide of aspartic acid, and glutamine, $COOH.CH_2.CH_2CH(NH_2).CONH_2$, the amide of glutaminic acid, play exceedingly important parts in the metabolism of certain plants.

Small amounts of urea, CON_2H_4, have been found in a number of plants (*e.g.*, spinach, cabbage, potato). Guanidine, or imino urea, $NH : C(NH_2)_2$, is represented in the amino acid, arginine, and has been found in the free state in the seeds of vetch.

APPENDIX II

SECTIONS ON PHYSICAL CHEMISTRY [1]

THE three states of matter are represented in plants. Cell wall materials, starch and other grains, and calcium oxalate and other crystals, are good examples of solid substances ; liquids are represented by aqueous solutions and fatty oils ; and a mixture of gases and water vapour occupies the intercellular spaces. As a result of this diversity a large number of problems exist concerning the physical heterogeneity of plants. In this appendix, however, we shall confine our attention to certain of the physical properties of solutions that affect the structure and behaviour of living cells.

A. The Properties of Aqueous Disperse Systems

The relations between metabolic products and aqueous solutions (*e.g.*, cell sap) are very variable. Thus very little affinity is shown towards water by fatty and ethereal oils, suberin, cutin, waxes, solid resins, and crystals of calcium oxalate and calcium carbonate. Another set of substances *imbibe* water and swell, but do not dissolve ; the process is reversible, and the swollen substance contracts on drying. As examples of such substances we cite cellulose (whether associated with pectic substances or with lignin), hemicelluloses, starch, and certain proteins (*e.g.*, the gliadins and glutelins of cereals). Unchanged inorganic salts and many metabolic products (*e.g.*, organic acids and their salts, sugars, many glucosides, tannins, albumins, peptones, etc.) dissolve in water, and are consequently found in solution in cell sap. We are primarily concerned at present with the properties of systems composed of substances dispersed in water.

Classification of disperse aqueous systems as coarse dispersions, colloid solutions, and crystalloid solutions. Particles of matter are said to be in a dispersed state when they are separated by distances that are large in comparison with their own linear dimensions. Insoluble substances can often be dispersed sufficiently finely in water for some time to pass before the effects of surface tension and gravity cause the dispersed particles to coalesce. We speak of such heterogeneous systems as *coarse dispersions*, when the dispersed particles are visible under the microscope, *i.e.*, when their mean diameter is greater than 100 $\mu\mu$ (where $\mu = 10^{-3}$ mm. and $\mu\mu = 10^{-6}$ mm.). A *suspension* is a coarse dispersion in which the *dispersed phase* is solid and the *continuous phase* liquid (*e.g.*, a suspension of clay in water). The dispersed particles will not pass through ordinary filter paper. In an *emulsion* both phases are liquid (*e.g.*, an emulsion of oil in water). There are other *coarse dispersions* besides suspensions and emulsions. Thus a gas dispersed in a liquid gives a *foam*, a solid in a gas gives a *smoke*, a liquid in a gas gives a *cloud*, and so forth. Coarse dispersions are not permanently stable. For instance, clay particles separate out from any suspensions, and, in oil-water emulsions, the oil globules coalesce and cream to the surface. The irregular motion termed Brownian movement confers temporary stability on a suspension or an emulsion. This movement is caused by the bombardment of the particles from all sides by the rapidly

[1] For further information concerning the subject-matter dealt with in this appendix, see Bayliss (*20*), Findlay (*88*), Gortner (*97*), Stiles (*288*).

moving molecules of the aqueous continuous phase, and may be observed in a suspension of gamboge in water viewed under the high power of a microscope. The presence of a third substance may increase the stability. Thus lead shot sink at once in water, but can be held in suspension in jelly. The stability of a heterogeneous system is enhanced when the continuous phase offers resistance to the movement of the dispersed particles.

A solution may be defined as a stable disperse system which contains particles of molecular dimensions dispersed in a solvent. Externally the whole system appears to be homogeneous. The dispersed solute particles pass through ordinary filter paper, and the particles themselves cannot be seen even under the highest power of the microscope. From this last fact it appears that the linear dimensions of the solute particles in a solution must be less than half the wavelength of the shortest waves in the visible spectrum (400 $\mu\mu$). Actually there is no definite border-line between the finer suspensions and emulsions, and true solutions. It is usual, however, to describe as solutions the systems that contain solute particles less than 100 $\mu\mu$ in diameter dispersed in water, whether these are ions, molecules, or micellæ.[1] For single molecules dispersed in water, *i.e.*, molecular or ionic dispersoids, much variation occurs in the size of the solute particle. The diameters of molecules of hydrogen (0·1 $\mu\mu$), sodium chloride (0·26 $\mu\mu$), glucose (0·7 $\mu\mu$), hæmoglobin (2·5 $\mu\mu$), and starch (5 $\mu\mu$), indicate the order of this variation in molecular dispersoids.

There is evidence that dispersed molecules may become hydrated, and then associate to form larger aggregates. As the diameters of the dispersed particles increase and approach 100 $\mu\mu$ the properties of the system tend more and more to resemble those of a coarse dispersion. Aqueous solutions are described as *colloid solutions*, or *hydrosols*, when the mean diameter of the dispersed particles is greater than $\mu\mu$ 1 and as *crystalloid* or *true solutions* when the mean diameter is less than 1 $\mu\mu$. That the line of demarcation is not sharp is indicated by the use of the term *semicolloid* to describe the properties of certain solutions in which the diameters of the dispersed particles range about 1 $\mu\mu$.

Thomas Graham (1861) discovered that very diffusible solutes can pass rapidly through parchment membranes, *i.e.*, they dialyze rapidly, while solutes that diffuse but slowly dialyze but slowly or not at all. He described as crystalloids the substances that dialyzed, when he found that, as a rule, they could be crystallized; and the substances that did not dialyze, and these generally resembled glue in being amorphous, he termed colloids. At the present day we do not distinguish between crystalloid and colloid substances, but between the crystalloidal and colloidal states, since many examples are now known of substances which dissolve to yield, under certain conditions, crystalloid solutions, and, under others, colloid solutions.

Although solute particles in a colloid solution pass through ordinary filter paper, it is possible to hold them back (*a*) in filter paper that has been treated with collodion or gelatine so as to decrease the average size of the pores, (*b*) in clay or porcelain filters, or (*c*) in collodion thimbles. The solute particles of crystalloid solutions pass through the pores of these filters or thimbles. Ostwald (*219*) describes methods for separating particles in crystalloid dispersion from those in colloid dispersion, by

[1] A *micella* or *micelle* is the name given to an ultramicroscopic structure formed by the physical association of two or more molecules, either like or unlike, or of molecules and ions. None of the associated molecules or ions lose their identity; hence a micella s not to be considered as a single chemical compound.

such *ultrafiltration*. The technique has important applications in biochemical practice.

In crystalloid solutions the dispersed particles are less than 1 μμ in diameter, and pass through a parchment membrane as well as through filter paper. Crystalloid solutions may be regarded as *molecular* or *ionic dispersoids* according as the dissolved substance is a non-electrolyte (for example : sugars, many glycosides, glycerol, and many simple ketonic, aldehydic, and alcoholic substances), or an electrolyte (for example : inorganic salts, and vegetable acids, fatty acids, and amino acids, and their salts). Owing to the inherent movement of the molecules or ions crystalloid solutions are permanently stable as physical systems.

In colloid solutions the particles are less than 100 μμ but greater than 1 μμ in diameter, and pass through filter paper but not through the pores of a parchment membrane. Colloidally dispersed particles may be separated from crystalloidally dispersed particles by *dialysis* (see fig. 65), or by *ultrafiltration*. That colloid solutions are physically heterogeneous is often indicated by their turbidity ; and even when they are absolutely clear liquids, the scattering of light which is shown when they are intensely illuminated from one side only (*Tyndall phenomenon*) demonstrates their heterogeneity. In microscopical examinations of the so-called Tyndall cone, the presence of dispersed particles in colloid solution is at times indicated by visible points of light which show active Brownian movement. A special apparatus, called the *ultramicroscope*, is used to make such observations (see *e.g.*, Bayliss *20*). Colloid solutions are heterogeneous physical systems whose properties are intermediate between those of a true solution and a coarse dispersion. The conditions that determine the stability of colloid solutions (*cf.* coarse dispersions) will be discussed below. We note here that the properties of the colloid solution formed by shaking clay with water and then filtering, bear on problems of the physical state of soils (chap. V), and that there are very many metabolic products which dissolve in water to give hydrosols. Examples of these products are the proteins, many polysaccharides (*e.g.*, starch, inulin, glycogen), gums, and tannins. Peptones and the salts of fatty acids (*e.g.*, soaps) may be mentioned as semi-colloids.

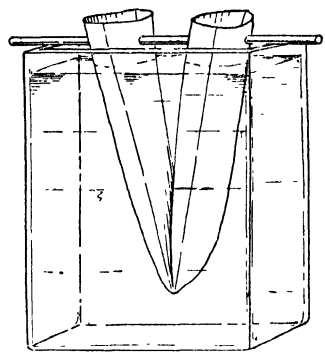


FIG. 65. When it is free from defects, parchment tube, looped, pierced with a glass rod, and set up as shown in the diagram, makes an effective dialyzer. It should be filled with the solution under examination, and water is placed in the containing vessel. Particles in crystalloidal dispersion will diffuse across the membrane, and those in colloidal dispersion will be held back. So as to maintain a diffusion gradient for the former, the water in the containing vessel should be changed periodically. If the object of the experiment is to free the colloid solution from crystalloidally dispersed particles, water running at a slow speed should be used. The glass rod should then be raised so as to prevent the leakage of liquid in and out of the parchment tube.

Hydrosols have been classified in two groups, the *hydrophobe systems*, and the *hydrophile systems*, or *emulsoid hydrosols*.

Hydrophobe systems. The generic term *hydrophobe* implies that the dispersed particles possess but little or no affinity for water. We may describe by the specific term *suspensoid hydrosol* any solution that contains in dispersion solid particles less than 100 μμ but greater than 1 μμ in diameter. Inorganic suspensoid hydrosols of gold, silver, arsenious sulphide ferric hydroxide, clay, etc., are readily prepared (see Onslow, *216*). They are clear non-viscous liquids, but show the Tyndall phenomenon, and particles in Brownian movement can be detected with the ultramicroscope. Suspensoids owe their stability mainly to the fact that the dispersed particles are electrically and similarly charged, and thus mutually repel one another. Either positive or negative charges may originate through direct ionization or through the adsorption of ions of the dispersed particles; and negative charges may also result from contact with water, which possesses a high dielectric constant. The Brownian movement of the particles also favours stability.

The presence of charged particles may be demonstrated by imposing an electromotive force on a sol. Thus for example, the negatively charged particles of a metallic sol may be made to move towards the positive pole. This movement of colloidally dispersed particles under an electromotive force is termed *cataphoresis*.

The colloidally dispersed particles in suspensoids are precipitated by the ions of opposite sign contained in added electrolytes. The flocculation of negatively charged particles by the addition of bivalent ions of calcium is one of the advantages that accrued from liming certain wet clay soils. The precipitating power of an ion increases with its valency. Thus it has been estimated that for positively charged particles, the precipitating powers of PO_4''', SO_4'', and Cl', are in the ratio 1,000 : 35 : 1. It is characteristic of suspensoids (*cf.* the behaviour of emulsoids) that their stability is destroyed by small quantities of electrolyte. Precipitation is virtually irreversible, drastic treatment being required to bring about redispersion.

Hydrophile systems (or emulsoid hydrosols). Certain molecules or micellæ having an affinity for water (hence the term *hydrophile*), imbibe water to give liquid particles of mean diameter between 1 μμ and 100 μμ, which become dispersed in the solvent. The system is described as an emulsoid hydrosol. Most of the colloid solutions occurring in plants (*e.g.*, cell sap containing proteins, tannins, or inulin) are of this hydrophile or emulsoid type. Moreover, emulsoid hydrosols are readily prepared from certain plant products, *e.g.*, gums, starches.

Emulsoid hydrosols are turbid and show the Tyndall effect. They cannot, as a rule, be resolved under the ultramicroscope. Doubtless the dispersed particles are in Brownian movement, which, together with the electrical properties and the viscous state of emulsoid sols, will favour stability.

Cataphoresis experiments indicate that the dispersed liquid particles are often electrically charged. Dispersion will be favoured by the mutual repulsion of particles charged with the same sign. As a class, however, emulsoid sols differ from suspensoids in not being precipitated by small amounts of electrolyte. There is a factor promoting stability which dominates the electrical properties. This is probably the high affinity for water possessed by dispersed particles, since the addition to an emulsoid hydrosol of a sufficient amount of a substance having a higher affinity for water than the dispersed particles leads to the dehydration of these particles, and, consequently, to the separation of the dispersed solute as solid particles. Thus in strong solutions of salts (*e.g.*, from half to fully saturated solutions of ammonium or sodium sulphate) solid particles

appear. These may be collected by filtration through ordinary filter paper, or by decanting the liquid above the solid mass that is formed by the settling of the particles either under the action of gravity or under the action of centrifugal force applied in a centrifuge. Ethyl alcohol is another dehydrating agent which causes precipitation in emulsoid sols, regardless of the molecular structure of the dispersed particles.

The precipitates that form on simple dehydration of emulsoids redissolved when the conditions again favour dispersion (*cf.* the irreversible precipitation of suspensoids by weak electrolytes). Conceivably this precipitation and redissolving by changes in the salt concentration of the continuous phase may play a part in certain cell events. Thus certain freezing injuries from which plants suffer in the field and in cold storage have been attributed to the precipitation of colloids following the increase in concentration in salts which occurs when ice crystals separate in cell sap. Possibly, however, the mechanical injury caused by the separation of ice crystals affords a better explanation.

Emulsoid hydrosols are more *viscous* than pure water, and their *viscosity* increases with increase of concentration or with lowering of temperature. Conversely, the viscosity is reduced by dilution or by warming. Emulsoid sols owe their stability chiefly to their high internal friction. This opposes agglutination and the settling of dispersed particles under the action of gravity. In many emulsoid hydrosols (*e.g.*, gelatin, agar-agar, soap, starch, and silicic acid) a progressive increase in viscosity results in a change of physical state.[1] The sol sets and becomes a gel. *A gel may be defined as a more or less rigid emulsoid system.* Whether a hydrophile colloid takes the form of a sol or a gel depends principally upon its concentration and the temperature. Thus the viscosity of a solution of gelatin in warm water increases as the temperature is lowered, and sooner or later the sol sets to a hydrogel. It appears, also, that the salt composition and the hydrion concentration may affect the viscosity of a hydrophile colloid, and, consequently, play a part in governing sol $\rightleftharpoons$ gel transformation. A further point to note is that gelatin and other gels may sometimes be converted into sols by mechanical agitation.

Inasmuch as a gel can oppose a shearing stress, *i.e.*, a stress which tends to distort the gel without increasing or decreasing its volume, it possesses rigidity. If the stress is below a certain limit, the gel will return to its original form when the stress is removed. If the stress exceeds this limit, the gel flows, and, when the stress is removed, the gel remains in its distorted condition, *i.e.*, the gel is plastic. If the value of the stress that causes plastic flow is very low it becomes difficult to distinguish such a highly plastic gel from a viscous sol; for the essential distinction between a solid and a fluid is that whereas a solid will come to a position of equilibrium under a shearing stress, and flows under the stress only if this exceeds a certain finite value, a fluid cannot resist a shearing stress, however small this may be. Different fluids yield at very different rates to a small shearing stress. The more viscous a fluid is, the more slowly does it yield. Clearly, in limiting instances, it becomes difficult to distinguish between a highly viscous fluid and a very plastic solid.

Mention may be made here of an interesting phenomenon, termed

[1] Unfortunately, little is known about sol $\rightleftharpoons$ gel equilibria in naturally occurring proteins, lecithins, components of mucilage and pectic substances, etc. Hydrogels have not yet been prepared from hydrosols of inulin, glycogen, dextrin, araban, xylan, the complex acids in gums and resins, tannins, and native albumins and globulins.

hysteresis, shown by many gels. The use of this term in colloid physics can best be explained by means of an example. Agar-agar by imbibing water swells in the cold. On heating to about 100° C., the swollen gel changes to a sol. On cooling, the sol does not re-set to a gel until a temperature of 35° C. has been reached. There is thus a difference of nearly 70° C. between the melting point of agar gel, and the setting point of agar sol. Gelatin shows a similar but slighter lag.

Enormous pressure is required to squeeze water out of a set hydrogel. It has, therefore, been suggested that when a sol turns into a gel, what was previously the continuous phase, viz., the more dilute solution, becomes the dispersed phase, while the previously dispersed liquid droplets of concentrated solution cohere to form the continuous phase (fig. 66).

Solid substances (*e.g.*, gelatin) that can form hydrogels, and plant organs (*e.g.*, dry seeds, or a piece of dry stipe of Laminaria) containing such substances, imbibe water and swell. As the volume of the swelling substance or organ increases,[1] great *imbibitional pressures* are developed. For instance, peas placed with water in a metal cylinder can in swelling be made to lift a weighted piston. The imbibitional capacity of hydrogels is affected by the salt composition and the hydrion concentration of the medium in which swelling occurs. It appears also that in many gels the imbibitional capacity decreases with time. It has been suggested that this decrease may play a part in the phenomena that lead to the senescence of cells.

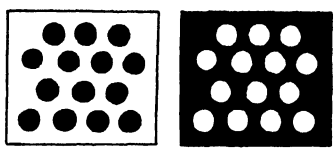


FIG. 66. Diagram to illustrate the reversal of phases during sol $\rightleftharpoons$ gel transformations. If the black represents the more concentrated solution, and the white the more dilute, A represents a hydrosol, and B a hydrogel.

One of the most characteristic properties of hydrogels is that they tend to contract and in doing so liberate aqueous solutions. Thus the liquefaction of agar slopes is a well-known phenomenon, and is described as *syneresis*. It has been suggested that syneresis may play a part in the secretory activities of cells. There is as yet no evidence, however, that plant protoplasm contracts spontaneously.

B. Phenomena Associated with Surfaces

Three obvious phase boundaries are seen when a vacuolated plant cell is mounted in an aqueous solution, and examined under a microscope: viz., those between (*a*) the cell wall and the aqueous solution,[2] (*b*) the protoplasm and the cell wall, and (*c*) the protoplasm and the cell sap in the vacuole. Furthermore, hydrosol or hydrogel systems are present in cell walls, protoplasm, and cell sap. This statement implies that as a result of the colloidal dispersion of metabolic products vast internal surfaces develop during the growth of plant cells. To illustrate this fundamental fact let us suppose that a cube of metallic gold with edge of 1 cm., and therefore having a total surface of 6 sq. cms., is subdivided to the limit of ultramicroscopic visibility (*i.e.*, into cubes the edge of each of which is 100 $\mu\mu$ in length).

[1] The total volume of water plus swollen gel is, however, less than that of the original volume of water plus solid. It is not known whether this contraction and the simultaneous liberation of heat possess biological significance.

[2] In a tissue of a higher plant, however, the water-saturated wall of a parenchymatous cell is exposed to the moist air of the intercellular space systems.

The total surface area of the 10^{18} cubes so formed, would then be 600 sq. metres. It has also been calculated that a surface of several thousand square metres would be developed by dispersing a cubic centimetre of dry starch so as to form a molecular dispersoid.

As we proceed in physically heterogeneous aqueous systems from the dimensions of particles found in coarse dispersions (suspensions and emulsions) to those in colloid and then to those in crystalloid solutions, the specific surface increases enormously. Phenomena associated with surfaces are therefore of great interest to physiologists.

The properties of the phase boundary between a liquid and gas or vapour have been much studied and the results indicate how other interfaces may best be considered. The forces of cohesion exerted between molecules in a liquid are very high. In the body of a liquid this force will act equally on every side of every molecule. At the phase boundary between liquid and gas or vapour, however, this force of cohesion does not act on the molecules from above. Consequently, although there are other forces that may cause molecules to leave surface layers, these layers behave as if they were in a state of tension. It is easy to demonstrate that soap films can be stretched. This stretching can be effected by blowing a soap bubble, or by exerting a pull on a moveable arm of a rectangular frame that encloses a soap film. The stretched film of the bubble and that in the frame contract when one ceases to blow the bubble or removes the pulling force from the arms of the frame.

Forces of cohesion may also create states of tension at the interfaces between solids and liquids, and between immiscible liquids. The general remarks which follow apply equally to these *interfacial tensions* and to the *surface tension* between a liquid and gas or vapour.

It is clear that areas under tension must be potential sources of energy as work must be done to increase the area under tension.[1] Now, there is a physical principle of universal application which states that the total free energy of any isolated physical system tends towards a minimum. For *surface energy* this tendency may be satisfied either by a diminution of surface area, or of surface tension.

The surface energy of a given liquid in contact with air can only be reduced by a diminution of surface area. Thus oil disperses in water and mercury scatters on glass as spherical droplets, and two clean droplets that touch will coalesce, *i.e.*, the liquid droplets always tend to assume the form (viz., a sphere) with the minimal surface area for a given volume of liquid.

When surface tension can alter, surface energy may be reduced by a diminution of surface tension, and this may happen without a change of surface area. It has been found that most solutes, other than inorganic salts, lower the surface tension between water and air, and that interfacial tensions between liquids are reduced by all solutes. It appears, therefore, that the free energy at interfaces may often be reduced by the migration

[1] But it must not be assumed that, if we increase the area of a surface, the total energy resident in the surface is equal to the work done in producing this increase in area. For, in order to stretch the surface at constant temperature, there must be an inflow of heat energy into the surface. The total energy of the surface therefore consists of two terms, and the term representing the heat entry makes a very important contribution to the total surface energy. Methods for determining surface tensions may be found in any manual of practical physical chemistry. The results are usually given in dynes per centimetre, or, what amounts to the same thing, in ergs per square centimetre of surface. The surface energy then, as is shown in books on thermodynamics, is equivalent to the maximum work available in an isothermal transformation.

of solutes to these interfaces. The formation of stable emulsions by shaking a fatty oil with water in the presence of alkali, provides a good illustration. Fatty oils always contain free fatty acids. These combine with the alkali to yield soaps, which lower the interfacial tension between the oil droplets and water, and hence migrate to the surface of the droplets. Consequently the free energy of these surfaces is reduced, and the tendency towards the diminution of the surface area by the coalescence of the liquid droplets is opposed, *i.e.*, the emulsion becomes stable. The formation of an insoluble film at the surface of an egg albumin solution in water provides another instructive example. This film owes its origin to the fact that egg albumin lowers the surface tension of water in contact with air. Consequently the albumin particles tend to migrate to the surface, and the concentration of albumin in the surface layers becomes so high that the albumin coagulates and forms a solid pellicle (*cf.* plasmatic membranes, p. 17).

An important consequence of the migration of substances that lower surface or interfacial tensions to phase boundaries is that the concentration of these substances becomes greater at these boundaries than in the body of the system. The process which causes such an increased concentration at a phase boundary is described as *mechanical adsorption*. We may thus state that soap becomes mechanically adsorbed at the surface of oil droplets dispersed in an aqueous medium, and that albumin becomes mechanically adsorbed at the phase boundary between air and an aqueous solution of albumin. Adsorption must be carefully distinguished from absorption. In adsorption there is a local concentration on a surface ; in absorption we picture the absorbed substance as uniformly distributed throughout the absorbing system.

The decolorizing of many liquids, for example, solutions of gentian violet, by animal charcoal is a simple way of showing that solids may act as *adsorbents*. Most of the quantitative investigations on adsorption have been on solid gas and solid liquid interfaces. Let us suppose that a mass M of a given adsorbent, such as a standard preparation of animal charcoal, is dispersed in solutions of different strengths of a single substance (*e.g.*, acetic acid) that reduces the interfacial tension between water and charcoal, and is consequently adsorbed by the charcoal. The equilibrium between the concentration of the solute in the main body of the solution and that on the surface of the adsorbent is given by the equation, known as *Freundlich's isotherm*—

$$x/M = aC^b,$$

where x is the mass of solute adsorbed, M the mass of the absorbent, C is the final concentration of the solute in the continuous phase, and a and b are constants. The constant b is always less than 1, and often about $\frac{1}{2}$, when, of course, $x/M = a\sqrt{C}$. This equation implies that the absolute amount of solute adsorbed increases with the initial concentration of the solution, and also with the mass of the adsorbent used. This experimental relation also indicates that, for a given mass of adsorbent, the relative amount of adsorption decreases as the initial concentration increases. Dilute solutions may be almost completely cleared of the solutes that can be adsorbed, whereas in stronger solutions, although more solute is actually adsorbed, there remains a residual amount in the continuous phase. The relative amount that remains unadsorbed increases as the initial concentration is raised.

If logarithms are taken of the terms in the adsorption equation we arrive at

$$\log (x/M) = \log a + b \log C.$$

Thus if an adsorbent has been adsorbing a solute from a solution, and itself remains unchanged (*i.e.*, M is constant), a straight line should be given when the logarithm of the residual concentration of solute in the solution at equilibrium is plotted against the logarithm of the amount taken up. Unfortunately, the giving of a straight line does not provide rigid proof that adsorption has been in progress, for processes, supposed not to be mechanical adsorption, appear also to be governed by Freundlich's isotherm.

Adsorbed molecules are never arranged at random on the surface of an adsorbent, but are orientated in a manner imposed by their own molecular structure and by the solvent properties of the adsorbent and of the medium surrounding it. Thus the molecules of salts of fatty acids adsorbed on the surfaces of dispersed oil drops in oil-water emulsions are *orientated* so that the hydrocarbon ends of the molecules are directed towards the oil, and the acidic ends towards the water. These adsorbed films may be only one molecule in thickness. Chemical groups that tend to dissolve in water are called *polar groups*, and those for which water has no affinity are called non-polar groups. The following are the polar groups of biochemical interest: $-OH$, $-CHO$, $-CONH_2$, $-SH$, $-NH_2$, $-COOH$, $-COOM$ (metallic salt), $-(H_2PO_4)$ (organic phosphate). Substances possessing polar groups will be hydrophilic (p. 15). Certain non-polar groups (*e.g.*, the long chains in fatty acids) are lipophilic, *i.e.*, will orientate towards fats or fat solvents.

Some workers regard adsorption as a purely physical process. Others, however, maintain that chemical forces, such as those of residual valency, always play an essential part in attracting and holding the adsorbed molecules. Purely mechanical adsorption should be independent of the molecular structure of the surface, and be solely determined by the reduction of interfacial tension. If chemical forces are in control, preferential adsorption may occur. This may be determined by the molecular structure, and possibly by the orientation, of molecules at these surfaces.

Many insoluble substances become electrically charged when they are dispersed in aqueous solutions, either by adsorbing hydrogen, hydroxyl, or other ions, or owing to the dielectric peculiarities of the solvent. Such electrically charged surfaces will be seats of free electrical energy, and this will tend to diminish. Hence these particles will tend to adsorb from the medium other particles, such as ions, carrying electrical charges of opposite sign. This type of adsorption is termed *electrical adsorption*, and it may play a part in the uptake of ions by plant cells. Electrical adsorption may be simply illustrated by experiments on filter paper. Since water possesses a high and filter paper a low dielectric constant wet filter paper is negatively charged, and takes up large amounts of electrically positive dyes such as night blue, but only small amounts of negatively charged dyes such as congo red. If the dielectric constant of the medium is reduced by adding alcohol to it, more congo red is adsorbed. It is probable that both mechanical and electrical adsorption play parts in these experiments.

Finally, we note that the outer surface of a whole plant exposed to moist air may be the seat of electrical charges.

C. Hydrogen-ion Concentration[1]

The total acidity and the hydrogen-ion concentration of acid solutions, and plant-sap. Plant sap is nearly always acid to litmus, and many tissues contain vegetable acids in high concentration. Free fatty and amino acids,

[1] See Small, J. *pH* and Plants: an introduction for beginners. London, 1946.

certain proteins, and aromatic and other acids, may also contribute to the total acidity of plant sap; and it must be remembered that a slight contribution is made by the carbonic acid that is produced when respiratory carbon dioxide dissolves in the cell sap. Titrations with standard alkali yield data for expressing the acidities of the sap of different plants in terms of normality. It may in this way be shown that lemon juice is approximately a normal solution of acidic substances, and that tomato juice is approximately a decinormal acid.

In considering the acidity of soils, the physical state of cell proteins, the activity of enzymes, variations in the colour of anthocyanins, and other problems, investigators have found chief interest to reside not in the absolute amount of replaceable hydrogen in plant sap (*i.e.*, its total acidity), but in the *concentration of hydrogen ions* (for short, hydrion concentration [H·]) in the sap.

All acids are electrolytes. However varied their molecular structure may be, whether that of a simple mineral acid, an acid salt (*e.g.*, sodium dihydrogen phosphate), or a complex protein, all acids possess in common the property of becoming electrically dissociated in water and giving rise to positively charged hydrogen ions, and negatively charged anions:

$$HX \rightleftharpoons H\cdot + X'.$$

The equation indicates that when an acid is dissolved in water, an equilibrium becomes established between undissociated molecules and the ions into which the acid dissociates. For a given acid, it is clear that the hydrion concentration will in the first place depend upon the total acidity of a solution. Thus the hydrion concentration of a normal solution of a given acid will be considerably greater than that of a decinormal solution.

Equinormal solutions of different acids possess the same *total acidity* as determined by titration. Thus in order to neutralize V ccs either of normal hydrochloric acid or of normal acetic acid, V ccs of normal alkali must be added. But this does not mean that the hydrion concentration of these normal solutions is the same; for the hydrion concentration of an acid depends not only on its normality but also on its degree of dissociation at that normality.

Physical experiments have shown that there is much variation in the percentage dissociation of normal solutions of different acids,[1] *e.g.*, the degree of dissociation of normal hydrochloric acid is seventy-nine per cent., and that of normal acetic acid 0·45 per cent. There will therefore be more than 175 times the number of hydrogen ions in a normal solution of hydrochloric acid than in the same volume of a normal solution of acetic acid, *i.e.* [H·] of normal hydrochloric acid $> 175 \times$ [H·] of normal acetic acid.[2] Acids which give normal solutions of relatively high hydrion

[1] The dilution governs the percentage dissociation of a given acid. In general it may be stated that the more dilute the solution the nearer to completion does dissociation become. Thus, by diluting normal hydrochloric acid ten thousand times, the degree of dissociation is increased to ninety-eight per cent. The hydrion concentration will, however, decrease greatly (see footnote [2]).

[2] The *absolute hydrion concentration* [H·] is usually expressed as gram ions per litre. Let us suppose a condition that never actually occurs, viz., that an acid is completely dissociated in normal solution. Such a normal solution would contain 1 gram of hydrogen ions per litre, since a normal solution contains 1 gram of replaceable hydrogen per litre. Hence, normal hydrochloric acid, being only seventy-nine per cent. dissociated, will contain 0·79 gram ions per litre. This is usually expressed by stating that the [H·] of normal hydrochloric acid is $7{\cdot}9 \times 10^{-1}$, and that of normal acetic acid is $4{\cdot}5 \times 10^{-3}$. The [H·] of N/100 hydrochloric acid (see footnote [1]) will be 0·98/10,000 or $9{\cdot}8 \times 10^{-5}$. This is much less

concentration are described as strong acids (*e.g.*, hydrochloric acid), and those which give normal solutions of relatively low hydrion concentration as weak acids (*e.g.*, acetic acid). Both strong and weak acids may be present in the mixture of substances that contribute to the total acidity of plant sap. Clearly, therefore, the hydrion concentration of plant sap cannot be judged from its total acidity. Thus the hydrion concentration of lemon juice may be very different from that of normal hydrochloric acid, and that of tomato juice from decinormal hydrochloric acid.

Besides the total acidity and the percentage dissociation of the various acidic components at the concentrations in which they occur, what are termed *buffer substances* play an important part in governing the hydrion concentration of plant sap. The buffer substances that interest us are characterized by dissolving to give solutions (buffer solutions) that resist changes in hydrion concentration when acids are added to them. Thus the salts of weak acids may be described as buffer substances, for it can readily be shown that a much smaller increase in hydrion concentration occurs when a certain volume of a strong acid (*e.g.*, decinormal hydrochloric acid) is added to a solution of a salt of a weak acid (*e.g.*, sodium acetate) than would occur in the absence of the salt. This is because the hydrogen ions of the strong acid and the acetate ions set free by the dissociation of sodium acetate combine to form undissociated molecules of the weak acid (acetic acid).

$$CH_3COONa \rightleftharpoons Na\cdot + CH_3COO' \text{ (strongly dissociated).}$$

$$HCl \rightleftharpoons H\cdot + Cl' \text{ (strongly dissociated).}$$

$$H\cdot + CH_3COO' \rightleftharpoons CH_3COOH \text{ (feebly dissociated).}$$

The results of the experiments of Martin, and of Ingold and Armstrong, carried out in Small's laboratory, indicate that the salts of phosphoric acid and of malic and citric acids may play an important part in the buffering of cell sap. It is important to realize that the hydrion concentration of a plant sap that contains such substances may be much higher than it would have been in their absence. Buffers have been aptly described as *moderators* or *regulators* of acid reaction; and there is little doubt that buffering in cells is frequently of great significance. Cell substances possessing surfaces that adsorb hydrogen ions, and calcium ions when they combine with free acids (*e.g.*, oxalic acid) to form insoluble salts, may also be regarded as buffering agents. Small pointed out that whereas certain buffer substances (*e.g.*, phosphates) are absorbed from the soil, others are metabolic products, *i.e.*, metabolism may regulate the hydrion concentration of cell sap. Respiration by producing carbon dioxide and photosynthesis by removing carbon dioxide can be effective metabolic buffering processes in plant cells.

The hydrogen-ion concentration of neutral and alkaline solutions.[1] Pure water has a low but measurable electrical conductivity. It may be regarded as an extremely weak acid and base, seeing that only one molecule in about five hundred million is dissociated into hydrogen and hydroxyl ions.

$$H_2O \rightleftharpoons H\cdot + OH'.$$

It has been shown that when hydrogen ions and hydroxyl ions are present

than the [H·] of normal acid, because the number of grams of replaceable hydrogen is ten thousand times less in the dilute acid; for, as we have noted before, it is the total acidity that in the first place determines what the [H·] of a given acid will be.

[1] The sap of the higher plants is usually acid, but the solutions in the environment are frequently neutral or alkaline.

in the same solution (whether acid, alkaline, or neutral) at 22° C., the product of the concentrations of these ions (*i.e.*, $[H^{\cdot}] \times [OH']$) is a constant and equals 10^{-14} gram ions per litre.[1] Now, in a neutral solution $[H^{\cdot}] = [OH']$; hence the $[H^{\cdot}]$ and $[OH']$ will each be 10^{-7} gram ions per litre.

When a solution is made alkaline by the addition of hydroxyl ions, the $[H^{\cdot}]$ will decrease as the $[OH']$ increases, and the product $[H^{\cdot}] \times [OH']$ remains constant. Thus if $[OH']$ increases to 10^{x-7} (where x is a positive number), $[H^{\cdot}]$ will decrease to 10^{-7-x}. Clearly, as a solution becomes increasingly alkaline, the $[H^{\cdot}]$ increasingly diminishes. Hydrogen ions, however, will always be present even in very alkaline solutions, and it is possible, therefore, to express alkalinity in terms of the $[H^{\cdot}]$ of the solution. We may define an alkaline solution as an aqueous solution that contains less than 10^{-7} gram ions of hydrogen per litre. For example the $[H^{\cdot}]$ of N/100 sodium hydroxide is $1{\cdot}02 \times 10^{-12}$.

The $[H^{\cdot}]$ of an alkaline solution depends upon (*a*) the concentration of alkaline substances present (thus the $[H^{\cdot}]$ of normal sodium hydroxide is $1{\cdot}2 \times 10^{-14}$, *i.e.*, it is considerably less than that of decinormal sodium hydroxide), (*b*) the percentage dissociation of the dissolved bases (thus sodium hydroxide, being strongly dissociated in solution, is a strong base, and ammonium hydroxide, being feebly dissociated, is a weak base), and (*c*) the presence of buffer substances (*e.g.*, an alkaline salt of a weak acid, such as sodium borate) that resist the ionization of an added base into hydroxyl and other ions.

The measurement of hydrogen-ion concentration and its expression in terms of *pH*. The hydrion concentration of any solution may be directly measured with the hydrogen electrode or the quinhydrone electrode (for details, see Small, *270*). The numbers that have already been given for the hydrion concentration of various acid and alkaline solutions have been arrived at by these electrical methods. In the range that interests us, the hydrion concentration always works out at a number multiplied by a negative power of 10. These awkward negative indices may be avoided by using a notation introduced many years ago.

If we let *pH* (a symbol, not *p* multiplied by H) stand for the logarithm of $(1/[H^{\cdot}])$, we may express every hydrion concentration in terms of *pH*. Thus, the *pH* of a neutral solution is $\log (1/10^{-7})$, that of an acid solution is $\log (1/10^{x-7})$ where x is a positive number, and that of an alkaline solution is $\log (1/10^{y-7})$ where y is a negative number. It follows that the *pH* of a neutral solution is 7, that of an acid solution is less than 7, and that of an alkaline solution is greater than 7. It should be carefully noted that as the acidity increases the *pH* decreases, while the *pH* increases as a solution becomes increasingly alkaline. It will be also seen in table XVIII that the *pH* of a strong acid of given normality is less than the *pH* of a weak acid of the same normality.

The *pH* values of many aqueous solutions containing mixtures of two or more substances, each in known concentration, have been determined by means of the hydrogen electrode. Clark (*61*) and others have tabulated the results and given instructions for the preparation of solutions of any desired *pH* values between one and twelve.[2] These are usually buffer solutions containing a salt of a weak acid. For example, a 0·908 per cent. solution of KH_2PO_4 has a *pH* value of about 4·5, and the *pH* of a solution of 1·19 per cent. $Na_2HPO_4.2H_2O$ is about 9·2. Buffer solutions of *pH* values inter-

[1] We note here that temperature has a slight effect on $[H^{\cdot}]$; *e.g.*, the ionization of water increases with temperature. Thus at 40° C., $[H^{\cdot}] \times [OH] = 10^{-13.62}$.

[2] Standard buffer solutions may be bought from several firms in this country.

TABLE XVIII. *pH values of hydrochloric and acetic acids and of sodium hydroxide at different normalities.*

Normality.	Hydrochloric Acid.	Acetic Acid.	Sodium Hydroxide.
N	0·10	2·37	14·05
N/10	1·07	2·87	13·07
N/100	2·02	3·37	12·12
N/1000	3·01	3·87	11·13
N/10000	4·01		

mediate between these two extremes may be obtained by mixing the two solutions in different proportions. For instance, measurements with the hydrogen electrode show that the solution obtained by adding 9·90 ccs of the solution of the alkaline sodium salt to 0·10 ccs of that of the acid potassium salt has a *pH* value of 8·17, while that obtained by adding 9·90 ccs of the solution of the acid potassium salt to 0·10 ccs of that of the alkaline sodium salt has a *pH* value of 4·98. Buffer solutions are not produced when pure salts are dissolved in water, for the addition of a drop of acid or base shifts the *pH* by several points. But the mixture of the acid potassium salt and the alkaline sodium salt shows strong buffer action in a range of *pH* that is of great interest to botanists.

If we are furnished with standard buffer solutions it is a simple matter to determine by means of the *indicator method* the *pH* of expressed plant sap, soil solution, or any other liquid.

A fact that is familiar to all who have titrated acids with bases is that the end points with different indicators are not exactly the same. The reason for this is that different indicators change colour at different *pH* values. For example, methyl-orange changes from pure red to pure yellow between the *pH* values of 2 and 5, while phenolphthalein does not become rose tinted until a *pH* value of 9 is reached. Further, a given indicator, such as methyl-orange, is variously tinted in solutions of *pH* values that are intermediate between those of solutions in which the colours are pure. Thus methyl-orange is orange-red at *pH* 3 and orange at *pH* 4. The market at the present day offers a wide choice of indicators that show a gradual change of colour over various narrow ranges of *pH* (see table XIX).

TABLE XIX

Name of Indicator	Colour Range	Range of *p*H.
Thymol-blue (acid range) .	Red-yellow	1·2–2·8
Brom-phenol-blue . . .	Yellow-purple	3·0–4·6
Brom-cresol-green . . .	Green-blue	3·8–5·4
Methyl-red	Red-yellow	4·2–6·3
Brom-cresol-purple . .	Yellow-purple	5·2–6·8
Brom-thymol-blue . . .	Yellow-blue	6·0–7·6
Phenol-red	Yellow-red	6·8–8·4
Thymol-blue (alkaline range) .	Yellow-blue	8·0–9·6

A hypothetical example will show how the indicator method may be used to determine the *pH* of any liquid. Let us suppose that a yellow colour results when a few drops of thymol-blue are added to the liquid. This would prove that the *pH* lies between 2·8 and 8·0. We might then proceed to narrow the limits in several ways. Let us assume for the

sake of simplicity that brom-phenol-blue and brom-cresol-green are respectively coloured purple and green in the liquid. The *pH* of the liquid would then be in the neighbourhood of 4. By carefully observing the necessary precautions (see Small, *270*), we may now determine the value of the *pH* to 0·1. The principle governing this final step in the indicator method is that the *pH* of the liquid under experiment will be the same as the known *pH* of a buffer solution that gives the same tint with brom-phenol-blue, brom-cresol-green, or some other indicator that serves over the *pH* range 3·5 to 4·5.

The indicator method has been adapted by certain investigators (*e.g.*, Small, *loc. cit.*) for the direct determination of the *pH* of plant tissues. Various indicators are applied externally to sections of plants, and for each indicator the *colour* that develops is considered in relation to the colours given by other indicators.

Anthocyanins have been described as natural indicators, since many anthocyanins change colour when the *pH* of the solutions in which they are dissolved alters. In recent years it has been shown, however, that the effect of co-pigments at times dominates that of hydrion concentration upon the colour of these pigments (p. 439).

The buffer capacity of solutions and the buffer index. Buffer systems resist changes in *pH*. Thus by using a suitable indicator (*e.g.*, brom-phenol-blue) we can show that a drop or two of dilute acetic acid will cause a considerable diminution in the *pH* of pure water, but that several cubic centimetres of the acid must be added to bring about the same shift in a molar solution of sodium acetate.

The *buffer capacity* of cell sap, soil solution, or any other liquid, can be expressed by a number, the *buffer index*. A solution has a buffer index of unity when the addition of one gram-equivalent of a strong acid or alkali shifts the *pH* of one litre of the solution through one unit. In general it may be said that the amount of shift depends upon the nature and concentration of the buffer. Comparative values of the buffer capacities of different solutions may be obtained by determining with the hydrogen electrode or with the aid of indicators, the number of gram-equivalents of acid (or alkali) that must be added to equivalent amounts of a solution to produce unit change in *pH*. In this way buffer index values are obtained for solutions of known composition, cell sap, etc. (for details, see Small, *loc. cit.*). Experiments have shown that for each buffer system there is a definite range of *pH* over which significant buffer action is shown. Of the substances present in cell sap, it appears that phosphates and bicarbonates show marked buffer action in acid solutions of *pH* greater than 5, and that the salts of vegetable acids are the chief contributors to the buffer action of the more acid saps.

D. The Diffusion of Dissolved Solute Particles

The fact that solutes diffuse in liquid systems is easily demonstrated by placing a solution containing a coloured solute (for example, copper sulphate or eosin) on a gel of ten per cent. gelatin. The use of a gel precludes convection currents. Experiments have shown that solute molecules and ions diffuse from regions of higher to regions of lower concentration until the concentration becomes uniform in the body of the solution.[1] For a given substance the average rate of diffusion over a

[1] We recall, however, that at equilibrium the concentration at interfacial boundaries may differ from that in the body of the solution (p. 458).

distance L from a region where the concentration is c_1 to another where the concentration is c_2 is proportional to $(c_1-c_2)/L$. This rate increases directly with increase of temperature.

Different substances diffuse at different rates, under the same external conditions. Acids and alkalies (strictly hydrogen ions and hydroxyl ions), have the highest rates of diffusion ; then come the component ions and molecules of salts. Among the non-electrolytes, the rate of diffusion decreases as the complexity of the molecule increases. For example, glycerol diffuses more rapidly than cane sugar, and the dispersed solutes in colloid solutions of polysaccharides, proteins, tannins, etc., diffuse very slowly.

The presence of a non-electrolyte, *e.g.*, cane sugar, may appreciably reduce the rate of diffusion of organic solutes in water. In colloid solutions the rate is still further reduced ; consequently the rate of diffusion of a given substance may vary in different parts of a living cell.

Special problems arise when we consider liquid systems in which membranes akin to cell walls and protoplasts form a part. We recall that Graham classified solutions as colloid and crystalloid on the basis of the relative permeability of parchment and certain other membranes to different solutes. Only water can pass across a parchment membrane which separates two colloid solutions, but solute molecules also will pass when crystalloid solutions of electrolytes or non-electrolytes are used. For a single solute, or for two or more solutes that do not combine, diffusion of the solute molecules of a crystalloidal solution continues until the concentration of the various solute particles becomes the same throughout the system, *i.e.*, the final state is the same as that which is attained when the membrane is not present, although, of course, equilibrium is reached more slowly. For example, when a solution of glucose is placed inside a permeable parchment membrane (fig. 56), and a solution of sodium chloride outside the membrane, glucose molecules diffuse out (*exodiffusion*), and sodium and chlorine ions diffuse in (*endodiffusion*), until the concentrations of glucose and of sodium and chlorine ions become the same inside and outside the membrane.

The systems that give rise to what are termed *Donnan-equilibria* [1] are of biological interest. Let us consider a simple instance, supposing that inside a membrane there is a colloid solution of an electrolyte LX (L being a colloid cation), and outside a crystalloid solution of a salt MX, having the common anion X′. In this system equilibrium cannot be reached by the migration of the diffusible ions M· and X′ until each of these ions is distributed in equal numbers on the two sides of the membrane, because such a distribution would leave free electrical charges on the indiffusible ion L·. As would be expected experiments have shown that X′ passes in to a greater extent than M·. At equilibrium it appears that the product of the concentrations of the diffusible ions (*i.e.*, M· and X′) inside the membrane is equal to the product of the concentrations of these ions outside.

Conditions are much more complex, even in the simplest systems that interest biologists. Indiffusible cations and anions, for example protein ions, are present in the cell sap ; and in the environment and cell sap there occur in great variety metallic and acidic ions capable of endodiffusion or exodiffusion. Furthermore it must be remembered that hydrogen and hydroxyl ions produced by the ionization of water can diffuse across membranes. For example, in a complex system the presence of an indiffusible anion inside a membrane might result in the passage from a

[1] For clear accounts of Donnan-equilibria see Gortner (*97*) and Stiles (*291*).

solution of mineral salts outside of cations in excess of anions, with compensating exodiffusion of cations (see p. 88). If hydrogen ions were among those passing out, there would be a tendency (which might not become manifest owing to buffering) for the acidity of the solutions both inside and outside the membranes to change (see p. 88). It is highly important to realize that at equilibrium, however it might be brought about, diffusible ions would be unequally distributed on the two sides of the membrane. The concentrations and ionic charges of the indiffusible ions operate in controlling this distribution. For a given diffusible ion there may occur diffusion against a concentration gradient to the inside of the membrane, which contains the indiffusible ion. On the other hand, diffusion may cease when the concentration of a certain diffusible ion remains greater outside the membrane.

Other systems of great interest to biologists are those in which the

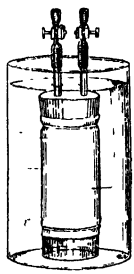


FIG. 67. A strong solution of tannin is placed inside the parchment membrane, and a weak solution of ferric chloride outside. The latter is crystalloid, and the former colloid. Ferric chloride diffuses across the membrane, and combines with tannin to form an ink. Such removal of the diffusing solute maintains a diffusion gradient, and, if excess of tannin is used, the outside solution finally becomes free from ferric chloride. McCullagh performed a similar experiment, but removed the diffusing solute by adsorption. She placed dilute acetic acid outside the membrane, and a suspension of animal-charcoal inside.

diffusing solute, after passing across a membrane, either combines chemically with (fig. 67) or is adsorbed on the surface of another substance inside the membrane. In either system, when the substance inside the membrane is relatively in excess, a concentration gradient will be maintained until the diffusing solute is entirely removed from the system. Even with relative excess of the diffusing solute, the final concentration of free solute molecules would be less than would have been found in the absence of the substance with which it combines chemically, or on which it is adsorbed, after endodiffusion.

Parallel systems occur in living plants. For example, during growth, newly formed cells by simple absorption remove diffusible nutrient substances, and thereby maintain diffusion gradients. When adsorption occurs as well, the gradients are steepened. Diffusion is also promoted when the diffusing substance undergoes metabolic change at the end of its path. Important examples are cited elsewhere (p. 93) of equilibria which, owing to metabolism, are never attained.

E. Osmotic Pressure

Early in the last century it was observed that sporangia of salt-water algæ increase in volume and burst when placed in fresh water. This observation led to investigations on the conditions that govern the passage of water across membranes. It was found that a hydrostatic pressure is always temporarily developed when a parchment membrane or animal bladder, enclosing a solution, is placed in pure water (see figs. 68 and 69). The term *osmotic pressure* was introduced to describe the maximum or equilibrium value of this hydrostatic pressure produced by osmosis.

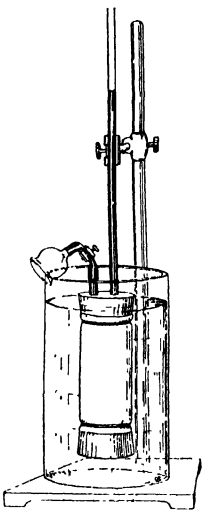


FIG. 68. The development of hydrostatic pressure as a result of the passage of water across a parchment membrane containing a strong solution of cane sugar, leads to a rise in the level of this solution.

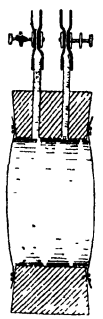


FIG. 69. The development of hydrostatic pressure as a result of the passage of water across a closed parchment membrane containing cane sugar, leads to an increase in turgor, and finally the membrane bursts.

Quantitative work was performed with a variety of solutes and membranes, and suggestive results were obtained. For instance, it was found that for a given solute and membrane, the osmotic pressure was governed by the initial concentration of the solution inside the membrane. It appeared, however, that the permeability of a membrane to the dissolved solute was also an important governing factor. Experiments with membranes of animal bladder showed that, at the outset, water tends to pass in, while the outward diffusion of solute particles tends to equalize the concentrations of the solutions on the two sides of the membrane.[1] The

[1] The membranes in living cells are probably not perfectly semipermeable for any solutes of crystalloid solutions, so the existence of the two tendencies mentioned above must always be kept in mind when considering the relations of living cells towards water and solutes.

fundamental fact was thus apprehended that a membrane permitting the passage of water only, *i.e.*, a perfect *semipermeable membrane*, must be employed in order to measure the true osmotic pressure of a solution.

It was known that parchment or animal membranes are semipermeable towards colloid solutions, but chief interest resided in the osmotic pressure of crystalloid solutions, towards which such membranes are permeable. Traube (1867) made the important discovery that membranes of copper ferrocyanide are semipermeable towards many crystalloid solutions. Traube's artificial cell may be prepared by placing a crystal of copper chloride at the bottom of a vessel containing a five per cent. solution of potassium ferrocyanide. There is thus produced a strong solution of copper chloride within a semipermeable membrane of copper ferrocyanide, outside of which is a weak solution of potassium ferrocyanide. The two solutions remain quite distinct, but water passes from the outside into the strong copper chloride solution. This is indicated by the increase in the volume of the solution inside the membrane, which is stretched and may break. It is, however, at once repaired by the production of more copper ferrocyanide. Odd-looking growth forms are often produced.

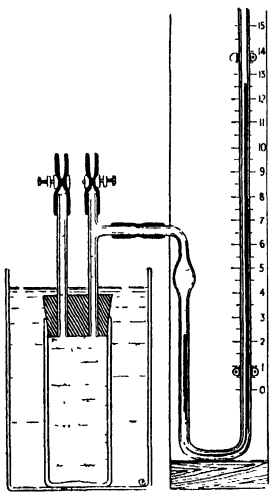


FIG. 70. Apparatus for measuring osmotic pressure. The solution is placed in a porous pot impregnated with copper ferrocyanide, and pure solvent is placed outside. The pressure is measured by means of the manometer, and the osmotic pressure is the maximum pressure observed.

Pfeffer applied this knowledge when he prepared a rigid semipermeable membrane for the measurement of osmotic pressure. Pfeffer placed a solution of copper sulphate inside and of potassium ferrocyanide outside a porous pot. Both solutes diffused into the capillaries of the pot, and, on meeting, combined to form copper ferrocyanide. The copper sulphate did not diffuse to the outside, nor did the potassium ferrocyanide reach the inside of the pot, *i.e.*, the porous pot impregnated with copper ferrocyanide in addition to being rigid, was semipermeable towards these crystalloid solutions. Using an experimental system similar to that illustrated in fig. 70, Pfeffer measured the osmotic pressure of crystalloid solutions of other solutes (*e.g.*, cane sugar) that did not penetrate the copper ferrocyanide membrane.

We may define *osmotic pressure* as *the equilibrium hydrostatic pressure produced by the osmosis of water into a solution placed in a perfectly semipermeable membrane, surrounded by pure solvent.* We note that according to this definition osmotic pressure does not cause osmosis but develops as a result of osmosis. When we say that a solution in a glass bottle has an osmotic pressure of x atmospheres, we mean that were it placed in a perfectly semipermeable membrane with pure solvent outside a hydrostatic pressure of x atmospheres would be developed.

Pfeffer's results, and those of later workers, have shown that the principal factor in determining osmotic pressure is the number of particles (whether

ions, molecules, or micellæ) present in unit volume of the solution.[1] Thus for solutions of any given substance it has been found that at constant temperature the osmotic pressure is approximately proportional to the percentage concentration in grams per unit volume of solvent. For example, in one experiment with cane sugar solutions of different concentrations the osmotic pressure in centimetres of mercury was 54 for one per cent. sugar, 102 for two per cent. sugar, 208 for four per cent. sugar, and 308 for six per cent. sugar. Solutions of different substances (other than isomers) in the same percentage strengths developed different osmotic pressures. For example, the following osmotic pressures were found for one per cent. solutions : cane sugar 47, dextrin 17, potassium nitrate 178, gum 7.

Now the molecular weight will in the first place determine the number of particles which will be present in a one per cent. solution of a substance. The larger the molecular weight, the fewer will be the number of molecules (or micellæ) present. It is therefore easy to understand why, for a given concentration, lower osmotic pressures are developed for colloid solutions of dextrin and gum—substances possessing high molecular weights—than for a crystalloid solution of cane sugar. Furthermore, when the solute is an electrolyte, the number of particles is increased as a result of the dissociation of molecules into ions. Thus the high osmotic pressure of the crystalloid solutions of potassium nitrate may be attributed to the small size of the molecule of this electrolyte, and to the dissociation of some of the molecules into potassium and nitrate ions.

These conclusions are of great importance in the consideration of the water relations of living cells (chap. IV, section A), for they permit us to infer that metabolism may bring about great changes in the osmotic pressure of the vacuolar sap in a given cell, without the introduction of fresh particles from outside. Thus the complete hydrolysis of a one per cent. solution of a condensate $(R)_n$ (*e.g.*, a polysaccharide) leads to an n-fold increase in the number of particles, and, consequently, to an n-fold increase in the osmotic pressure. Moreover, should the metabolism of a substance that is not an electrolyte lead to the production of an electrolyte (*e.g.*, an organic acid), a further increase in osmotic pressure will be brought about by the ionization of the metabolic products. On the other hand, the osmotic pressures of one per cent. solutions of simple sugars, amino acids, etc., decrease when condensations take place. The osmotic pressure becomes exceedingly low when the molecules or molecular aggregates reach colloid dimensions, and a further reduction occurs when a condensate goes out of solution and forms solid grains.

For solutions of different substances it may be stated that at constant temperature equal volumes of dilute solutions containing the same number of particles will develop the same osmotic pressure when placed in a perfectly semipermeable membrane with the pure solvent outside. We describe solutions as *isotonic solutions* when they have the same osmotic pressure. When solutions have different osmotic pressures, that with the higher is said to be *hypertonic*, and that with the lower *hypotonic* with respect to the other.

[1] Temperature is also a governing factor, but its effect on the osmotic pressure of cell sap is comparatively slight. For dilute solutions the relationship between osmotic pressure (P) and concentration (C) and the *absolute* temperature (T) may be summarized by the equation $P = RCT$ where R is the gas constant. This implies that the gas laws hold for dilute solutions, and that the osmotic pressure of a solution is equal to the gas pressure that the solute particles would exert if the solvent were suddenly annihilated, and the volume remained unaltered.

It has been calculated that the molecular weight in grams (one gram molecule) of any substance contains $6{\cdot}06 \times 10^{23}$ molecules. Equimolar solutions [1] will contain the same fraction of this huge number of molecules, and hence, for non-electrolytes, the same number of particles. And, broadly, it is true to state that experiments have shown that equimolar solutions of non-electrolytes are isotonic.[2] Thus $34{\cdot}2/a$ per cent. cane sugar (molecular weight = 342) and $18{\cdot}0/a$ per cent. glucose (molecular weight = 180)—where a would be 1 for a molar solution, 2 for a 0·5 molar solution, etc.—are isotonic. The osmotic pressure of dilute solutions of non-electrolytes may be approximately calculated from the relation arrived at by the application of Avogadro's law for gases to such solutions, viz., that a solution containing the molecular weight in grams dissolved in 22·24 litres will at 0° C. have an osmotic pressure of 1 atmosphere, which is equal to the pressure exerted by 76 cm. of mercury. For instance, 0·1 molar solutions of cane sugar, glucose, and other non-electrolytes, have, approximately, osmotic pressures of 2·2 atmospheres at 0° C.

Solutions of electrolytes have higher osmotic pressures than those of equimolar solutions of non-electrolytes. The osmotic pressure of a 0·1 molar solution of potassium nitrate (molecular weight 101) is considerably greater than 2·2 atmospheres; how much greater will depend upon the degree to which the molecules are dissociated into ions at this molar concentration. Taking a general case, let us suppose that in a given molar strength of solution x per cent. of the molecules of a dissolved electrolyte, MX, are dissociated according to the equation.

$$MX \rightleftharpoons M\cdot + X'.$$

Then, if the osmotic pressure of an equimolar solution of a non-electrolyte is P, that of the electrolyte will be $(1 + x/100)P$. Thus were eighty per cent. MX dissociated in a 0·1 molar solution, the osmotic pressure would be approximately $1{\cdot}8 \times 2{\cdot}2$ atmospheres. It should be noted that the degree of dissociation of a given electrolyte increases as the concentration is decreased. For potassium chloride solutions at 0° C., the percentage dissociation is 86 when one gram molecule is dissolved in ten litres of water, and 95 in a solution ten times more dilute.

For certain purposes a number termed the *isotonic coefficient* is conveniently used to indicate the relative magnitudes of the osmotic pressures of equimolar solutions of different substances. At the present day the osmotic pressure (P_2) of the solution of the substance under investigation is compared with that (P_1) of an equimolar solution of cane sugar.[3] As a standard

[1] The molecular weight in grams dissolved in one litre of solvent, is a weight molar solution, and in one litre of solution, is a volume molar solution.

[2] It should, however, be noted that the differential effects of several factors, *e.g.*, the mutual attraction of dispersed molecules, bring about differences when strong equimolar solutions are compared.

[3] Direct determinations of the osmotic pressure of cane sugar have been made with great care over a wide range of concentration (for table see Small, *271*). From the tabulated figures we can arrive at the osmotic pressures of solutions of known molar strengths of other substances. Direct determinations of osmotic pressures are difficult to make, so measurements are made of some other magnitude, such as the lowering of freezing point, raising of boiling point, or lowering of vapour pressure, that is a function of the osmotic pressure because it also depends upon the number of particles dissolved in unit volume. We can thus determine by experiment the molar strength, aM, of a solution of an electrolyte (say), that is isotonic with bM solution of cane sugar, and of an a_1M solution that is isotonic with a b_1M solution of cane sugar, etc. We thus arrive at the osmotic pressures

the isotonic coefficient of cane sugar is taken as 2; that of the other substance will then be $2P_2/P_1$. The isotonic coefficient of a non-electrolyte works out at 2 (*i.e.*, it is the same as that of cane sugar) when it is determined by physical methods. For electrolytes, however, higher values are obtained; how much higher depends on the molecular structure of the substance and the mode and degree of ionization at the concentration used. For example, in a 0·1 molar solution the isotonic coefficient of potassium nitrate was found to be just over 3, that of normal potassium sulphate approximately 4, and that of potassium citrate just over 5. Here the principal governing factor appears to have been the maximum number of ions that can be formed by the dissociation of a single molecule: for KNO_3 can give two ions, K_2SO_4 three, and $K_3C_6H_5O_7$ four. For any electrolyte, however, we recall that the degree of dissociation, *i.e.*, the percentage number of molecules that are dissociated, varies with the concentration. Consequently, the isotonic coefficient is not a constant that can be evaluated by measurements for a single concentration of a given electrolyte, but must be determined experimentally for each molar strength that is to be used. The numbers obtained from determinations of *isotonic coefficients by plasmolytic methods* represent the relative *plasmolytic powers* of equimolar solutions (p. 81). Cane sugar solutions are again taken as standards for comparison (see footnote, p. 271). There are several methods which may be used. For a given tissue we may find the molar strengths of solutions of different substances that cause fifty per cent. of the cells to become plasmolyzed. Or, we may first plasmolyze the cells in a solution of cane sugar of known molar strength, and then transfer the cells to solutions of known strengths of other substances. That solution in which the volume of the cell sap enclosed in the protoplast of the plasmolyzed cells remains unchanged, will be isotonic with the cane sugar solution that in the first place caused plasmolysis. Clearly, isotonic coefficients can be calculated from the results of such experiments.

Curved strips cut from the inflorescence stalk of the dandelion also provide suitable material for experiments. In the intact peduncle the epidermal layer is stretched and the turgid parenchyma of the cortex and pith are in a state of compression. Cut strips take on a curvature as a result of the release of tissue tensions. The epidermal cells contract and the parenchyma expand. Curvature increases in water or in weak hypotonic solutions, owing to the endomosis of water into the parenchyma. In hypertonic solutions exosmosis occurs, and curvature decreases. No change of curvature is seen when the external solution is slightly hypotonic with respect to the cell sap in the parenchyma, *i.e.*, when $P_e = P - T$ (see p. 77). Solutions of different substances that cause no change of curvature may be regarded as approximately isotonic. Or curved strips may be made less curved by immersing in a hypertonic solution of cane sugar, and then transferring to solutions of other substances. That solution in which the decreased curvature does not alter will be isotonic with the solution of cane sugar that brought about this decrease.

When the protoplasts in the cells act as truly semipermeable membranes, permitting neither the endodiffusion of the solutes in the bathing solution

of the electrolyte in solutions of different molar strengths, and can then compare the osmotic pressures of equimolar solutions of the electrolyte and of cane sugar, *i.e.*, get a number for the isotonic coefficient of the electrolyte.

Cane sugar solutions serve also for the determination of isotonic coefficients by plasmolytic methods because the molecules of this solute penetrate protoplasm very slowly.

nor the exodiffusion of the solutes in the cell sap, the isotonic coefficients as determined by plasmolytic methods will be the same as those determined by physical methods. It often happens, however, that the protoplast is not truly semipermeable towards the solute in the bathing solution, and the difference observed between the isotonic coefficients as determined by physical and plasmolytic methods, then serves as a valuable index of the permeability of the protoplast to the solute.

APPENDIX III

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